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PROCEEDINGS OF THE INTERNATIONAL CONGRESS OF PLANT SCIENCES

ITHACA, NEW YORK

AUGUST 16-23, 1926

Edited by B. M. DUGGAR

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CONTENTS, VOLUME 1

Page

Introduction v

Addresses before the Congress as a whole

F. A. F. C. Went 1

Erwin F. Smith 13

Papers presented before the various sections of the Congress

A. Agronomy 47

Tulaikov, 47; Arrhenius, 53; Love, 55; Borodin, 61; Kiesselbach, 87; Stadler, 107; Kirssanoff, 129; Hayes, 137; Leighty, 149; Holbert and Dickson, 155; Vavilov, 161; Vavilov, 167; Borodin, 171.

B. Bacteriology 175

Bergstrand, 175; Henrici, 185; Buchanan, 195; Waksman, 203; Issatschenko, 211; Issatschenko and Salimowska, 221; Clark, 225.

C. Cytology 243

Němec, 243; Seifriz, 251; Svedelius, 259; Taylor, 265; Levine, 271; Blackburn, 299; Heilborn, 307; Harper, 311; Cleland, 317; Allen, 333; Sax, 345.

D. Morphology, Histology, and Paleobotany 351

Went, 351; Buchholz, 359; Hoyt, 393; Florin, 401; Hutchinson, 413; Eames, 423; Wieland, 429; Svedelius, 457; Chamberlain, 473; Thompson, 481; Chodat, 487.

E. Ecology 497

Domin, 497; Conzatti, 525; del Villar, 541; Skottsberg, 565; Toumey, 575; Palmgren, 591; Rübel, 603; Du Rietz, 623; Nichols, 629; Gleason, 643; Szafer, 647; Borza, 655; Sernander, 663; Pearsall, 667; Du Rietz, 673; Tansley, 677; Cowles, 687.

F. Forestry 693

Serpieri and Pavari, 693; Howe, 701; Toumey, 713; Jonson, 729; Zon, 741; Rodger, 751; Munns, 757; Cajander, 769; Petrini, 779.

G. Genetics 787

Lehmann, 787; Sirks, 803; Savastano, 815; Tischler, 821; Blakeslee, 831; Malinowski, 833; Shull, 837; East, 889; Muller, 897; Eyster, 923; Demerec, 943.

FOREWORD

At a meeting of the Council of the Botanical Society of America in December, 1923, the writer had the privilege of recalling that no international reunion of botanists had occurred since the Brussels Congress of 1910. It was suggested that perhaps the time might be favorable for a consideration of a renewal of these established conferences. Contingent upon several circumstances referred to more explicitly in a later, brief record of arrangements (Volume 2), steps were taken by the Botanical Society of America looking toward the formation of a Provisional Organizing Committee. With prompt support from the American Ecological Society, the American Phytopathological Society, (subsequently from other American research societies in the plant sciences) and later with the cordial approval of British botanists primarily concerned with the unrealizable London Congress proposed for 1915, the Organizing Committee undertook its task.

With gratifying cooperation, and later with promised participation, on the part of those interested in these fields of research throughout the world, the International Congress of Plant Sciences (Fourth International Botanical Congress) was successfully convened under highly favorable auspices at Cornell University, Ithaca, New York, August 16–23, 1926. Aside from the promotion of international goodwill and fellowship, the declared purpose of the Congress was primarily the presentation of the results of research in the various aspects of the plant sciences. Invitation programs were arranged by the Program Committee for thirteen recognized sections, and accordingly the two extensive volumes of these "Proceedings" are essentially a record of the critical and research papers presented, including likewise, however, a brief register of attendance, of organization, of excursions, and of the general proceedings of the Congress as a whole and of the several sections.

Volume 1 includes two addresses before the Congress as a whole and most of the papers presented in seven sections: Agronomy; Bacteriology; Cytology; Morphology, Histology, and Paleobotany; Ecology; Forestry; and Genetics. Volume 2 will contain the papers in Horticulture, Physiology, Pathology, Pharmacology and Pharmaceutical Botany, Taxonomy, and Mycology, as well as general and special proceedings. No formal subdivision into the respective sections has been made in publication, but footnotes are intended to indicate before which sections primarily the various papers were presented. Such footnotes are admittedly incomplete and do not adequately indicate the extent to which related sections mutually cooperated in the programs arranged.

The pagination of the two volumes is consecutive, and separate brief indices and tables of contents are arranged. It has seemed impracticable to list so many

titles in the "contents," accordingly only authors are so listed, each index including for the particular volume titles of papers and key words in titles, as well as names of authors.

As editor and as general secretary I have several duties to perform. First, I would express profound regret at the delay occasioned in the publication of the Proceedings, responsibility for which the editor must assume, since an explanation of the many circumstances causing delay would be both unprofitable and out of place. Thanks are due the secretaries of sections for their difficult work in collecting the papers. I am particularly indebted to my associate, Dr. C. E. Allen, for his assistance in the editorial work on the sections Cytology and Genetics. For the Organizing Committee (later the Executive Committee) I would express deepest gratitude to members of the Program Committee and of the Committee on Local Arrangements for their highly efficient organization and work.

B. M. Duggar

INTRODUCTION

A BRIEF ABSTRACT OF THE OPENING REMARKS OF THE CHAIRMAN OF THE CONGRESS

L. H. BAILEY
Ithaca, New York

In opening the Congress on the evening of Monday, August 16th, the Presiding Chairman spoke briefly, among other matters, of the great advances that have been made in the plant sciences, and indicated particularly the discoveries of the present generation and the changed points of view resulting therefrom. Yet as to the kinds of plants he declared that we obviously cannot state whether more than half the forms inhabiting the world are known, suggesting thereby our very short and imperfect knowledge of the vegetation of the earth. Up to 1900 apparently more than a half million Latin names had been applied to the seed-plants; perhaps half or more of these names are synonyms or duplicates. In the succeeding quarter century nearly or quite 200,000 Latin names have been recorded or revived. Probably less than 10 per cent of the plants of the world are known to cultivation, although great numbers have been "introduced" or planted at one time or another; and the greater part of this small percentage is of small importance to the welfare of the race. The great activity in systematic botany in recent time and the descriptions of surprising numbers of new species indicate how imperfectly the flora of the globe is understood. The species that contribute the major supplies of food and fiber as cultivated subjects is probably less than one or two hundred.

Even of the recognized species relatively few have been thoroughly studied physiologically and genetically. Of the vegetable community we assuredly know much, and yet very little in comparison with the prospects we can ourselves vision. The science of botany is yet young in spite of its age, and virile notwithstanding the hoary tomes that fill our shelves. We therefore come to the Congress looking to the future and with our minds full of conquests.

The Chairman called attention to the comprehensive nature of the Congress as indicated by the program. The departments of knowledge in the broad field of botany, as represented in the sectional meetings, may be arranged in four departments for the better understanding of the audience.

He mentioned first a department represented by a single section on the program. It is (1) Bacteriology, the study of cells that are separate, independent organisms, the science that knows the minutest and yet the most powerful forms of life. This great subject was practically established by Cohn, and vastly broadened by the studies of Pasteur; and there are those in the audience who remember the discussions of what was called "the germ theory of disease," with much opinion in opposition, for some of us still lived in the time when sickness

was a judgment for personal sin. It is very recent that disease has been taken from the realm of mysticism and accounted a natural phenomenon amenable to scientific investigation.

He then passed to the department concerned with structures, organization, and processes within the complex plant itself. Here we have (2) Cytology, that knows the cell as the unit of the structure of plants. When Von Mohl, some 80 years ago, published his classic work on the cell and also gave us the great word protoplasm, a practically new subject was uncovered. At first a part of anatomy, so fundamental have recently been the discoveries that the separate science of cytology has programs and extensive literature of its own. (3) Histology follows, the science that deals with tissues; (4) Morphology, the study of the plant forms and their development; (5) the inclusive subject of Physiology, that investigates all life-processes and functions.

The third main department of plant knowledge concerns itself largely with plants as species or kinds. Here come (6) Genetics, the science of breeding as represented in genealogy and heredity; (7) Ecology, or the plant in relation to its associations and environment; (8) Mycology, the study of fungi; (9) Taxonomy, the classification of plants; (10) Paleobotany, the knowledge of the records of plants in the rocks.

The fourth department includes the great applications, as (11) Agronomy, the wisdom of the fields as represented in crops; (12) Horticulture, the cultivation of personal products represented in fruits, flowers, vegetables, and ornamentals; (13) Forestry, the growing of trees to make woods; (14) Pharmacognosy and Pharmaceutical Botany, the knowledge of drugs derived from vegetable sources; (15) Pathology, the vast field of plant ailments and diseases.

We therefore introduce to you, the Chairman said, fifteen departments for your attention and consideration, although they are otherwise arranged and stated in the program; and we are glad also to welcome the Association of Official Seed Analysts, who make much of our practice possible. We anticipate a week of great enjoyment and benefit.

PLANT MOVEMENTS[1]

F. A. F. C. WENT
Rijks-Universiteit, Utrecht, Nederland

Mr. President, Ladies, and Gentlemen!

The general public is inclined to look upon a plant as something stiff and rigid; though everyone knows that stems and twigs and leaves may bend under the influence of the wind, yet it is generally thought that there can be no question of independent movements, not only of such that will offer plants the possibility of locomotion, but also of such as may lead to new positions of the different organs. A little reflection, though, will carry one to the conclusion that this general opinion is wrong. Anyone who has seen flower buds or other buds opening knows very well that plants are able to carry out their own movements; and if he has seen the same phenomenon as a moving picture, when all these movements become accelerated, the old wrong opinion will vanish very soon.

It is rather curious, though, that even among biologists, who ought to have known better, the same opinion prevailed during many centuries. With this line of thought it can be understood that the movements of a plant like *Mimosa pudica*, precisely defined as *the* sensitive plant, were gazed at as a wonder. We now know that movements of this order are only an extreme case of a phenomenon which can be seen throughout the plant world, only in a less conspicuous form.

Generally speaking, only during the course of the 19th century have botanists come to the conclusion that plants have a power of movement of their own, as well as animals. Some of the lower forms have their own locomotion, which is wanting in the higher plants; but these latter are able to direct their stems or leaves or roots under the influence of the environment.

In most cases they can do so only as long as these parts are not yet fully developed, because these movements are the result of an unequal growth of two opposite sides of the organ. But this is not always so; in a certain number of cases adult parts can perform periodical movements. So it is, for example, with the sensitive plant, already mentioned, and with most of the "sleeping" plants. The leaflets of the wood-sorrel (*Oxalis*), for example, assume a vertical position at night, whereas they stand horizontally during the daytime. Here, as in the other cases of adult parts, the movement is carried out by a change in the permeability of the protoplasm on one side of the pulvinus of the leaf, leading to an expulsion of water out of the cells on that side and hence a change in their tension. In the course of this lecture we shall see that also with growing parts, perhaps, something comparable to this phenomenon may be observed.

[1] Invitation address before the International Congress of Plant Sciences, session of the Congress as a whole, Ithaca, New York, Aug. 18, 1926.

After this digression I go back to the middle of the 19th century. Scientists became more and more convinced of the unity of all life. Consequently there could not be a fundamental difference between the power of movement of animals and plants, although it might well be that the way in which these movements are carried out is quite dissimilar. Hence, the power of the higher animals, more especially man, to respond in a certain manner to external stimuli was supposed to exist also in plants.

Let us take a young plant of the common garden bean as an example. When this is placed in a horizontal position, the root bends until the tip is directed towards the center of the earth. The old explanation, given by Hofmeister, was that here gravitation acted in the same way as on a bar of sealing wax when this is somewhat softened by a small increase of temperature. But, of course, this explanation became impossible for the movement of the stem, which curves in the opposite direction; here a very complicated theory was given, which I will not repeat. But, more and more, the conception of gravitation acting as a stimulus found acceptance; here, to be sure, the same force gave very different reactions according to the part of the plant on which it was acting. It was evident that the response was not determined by the acting force but by the internal structure of the plant.

Slowly, the conviction became general among botanists that all these movements are to be regarded as a response to a force, acting as a stimulus. Perhaps, first of all, Frank came to this conclusion, afterwards also Sachs, but the man who strengthened the cause here was Darwin, or rather were Charles and Francis Darwin. In their famous book, "The Movements of Plants," they showed, for example, that a root is no longer sensitive to gravitation when the tip has been cut away. Hence, they concluded that perception of the stimulus of gravitation takes place in the tip, whereas the response is carried out by the more basal parts, which elongate unequally on both sides. I shall let the question stand, whether Darwin really did prove this assertion by his experiments; we certainly know now that in the main Darwin was right. After some altercation, which we may leave out of consideration, Darwin's idea was taken up by many German scientists, and there it was in the first instance Pfeffer and his pupils who worked out a theory of plant movements which up to the present day has had a great influence on the conceptions of many botanists.

These movements, then, were considered as being released by some external stimulus, in the same way as by opening a cock an engine driven by steam may be put in motion. Everyone knows that the energy through which this motion is produced has its source in the burning of coal. In the same way the internal energy produced by the respiratory processes in the plant would be the cause enabling the plant movements to be carried out, and the stimulus from the outside would be nothing more than the movement of the hand of the engine driver. No simple relation then would exist between the size of the movement and the quantity of energy supplied by the stimulus. We shall see that in this respect the investigations of these last years have given us a very different conception.

Another line of thought to which sometimes even the same investigators

adhere is that which is based on teleological conceptions. It is suggested that all plant movements are carried out with a certain design, that is, to bring the plant in the most favorable position in regard to its environment. Gravitation acting on the chief root would cause this to bend towards the center of the earth, thus fastening the plant in the soil. The lateral roots react very differently in regard to gravitation; they grow in a direction which makes an angle with the vertical. According to these investigators this is done in order that they may make use of all the nutritive substances of the soil. Sometimes it becomes rather difficult to prove that a certain movement means anything to the plant. Let us take, for example, the movements of *Mimosa pudica;* up to the present, nobody has succeeded in guessing their meaning. It has been suggested that herbivorous animals would be so frightened by the movements of the leaves the moment they touch them, that they would be deterred from eating them, or that the withered appearance of the plants would have this same effect. This does not look very probable; and nothing better can be said of the idea that the leaves would in this way throw off the raindrops, thus preventing their getting wet, in order that no fungus growth might take place. But even if all this were proved for the leaves of *Mimosa pudica*, then there are other plants with sensitive leaves where this sensitiveness is so insignificant that it certainly cannot serve any purpose.

But, after all, this manner of reasoning, which was so common among naturalists some 30 or 40 years ago, really carries us out of the domain of natural science. He who likes to philosophize in this way is free to do so, but he should not then call himself a naturalist. The only way to proceed for us botanists is to analyze the movements as far as possible, trying by well-guided experiments to get at a causal explanation of the way in which the living substance responds to external forces.

I think that during these last twenty years the beginning of such an analysis has been made, more especially in regard to one movement, that is, that of the light responses of plants. I do not mean to say that in other domains no good work has been done, for example, on geotropism, chemotropism, the movements of *Mimosa*, etc., but it certainly is not so complete as all the intensive investigations upon phototropism. For it is this term we use now-a-days when we want to designate the movements of plants caused by unilateral light.

Everybody knows this phenomenon, that plants cultivated in a room lighted by a window, react in such a way that their stems bend towards the light, whereas the leaves, so long as they are not yet old, place themselves at right angles to the rays of the light. Roots generally do not respond at all, it is only with some plants, such as the white mustard, that they bend away from the light. We call the movements of stems positively phototropic, those of the roots just mentioned negatively phototropic, whereas we call the leaves of, say, the garden nasturtium transversely phototropic. Also lower plants may react phototropically. I will only give two examples, one a liverwort, of which the leaf-like thallus shows transverse phototropism, whereas the rhizoids on the back react negatively to one-sided light; the other instance is the common labora-

tory fungus, *Phycomyces*. Concerning this fungus, Oltmanns has shown us, now many years ago, that the response is not so simple as it looks; for here the sporangiophores are either positive or negative according to the distance from the source of light. The farther away the plants stand, the more do they react positively, nearer the light we find an indifferent zone, and yet nearer Oltmanns found them to give a negative response.

A more detailed analysis of this phenomenon has been made by Blaauw, some twenty years ago. In order to understand this, it is necessary first of all to look a little further back. In Darwin's "Movements of Plants" it was first made clear that seedlings of grasses are excellent objects for the study of phototropism. Rothert thirty years ago confirmed this by a great number of very exact experiments, where he made use especially of seedlings of the oat plant. I may remind you that with oats, just as with other grasses, the seedling above the soil first appears as the so-called coleoptile, or cotyledon. This is a completely closed cylindrical organ, attenuated towards the tip. In the hollow of this coleoptile the first leaf develops, till at last it breaks through. The first leaf shows no reaction to light whatever, but the coleoptile is extremely sensitive till the moment when the first leaf begins to appear. We can say that these oat seedlings are sensitive even to some rays which our eyes cannot detect, and the sensitiveness is such, that it is a serious drawback to the investigation, since any light, however small be it, has an influence on the curvature. Gradually it has been found that the red light of a photographic lamp is not very dangerous in this respect, but we must always consider that it has some influence, however slight this may be.

Now let us place oat seedlings for a few seconds in unilateral white light and after this let them stand in the dark. Then a curvature may result, very distinct after 3 hours, which is the after-effect of the former illumination. Blaauw and Fröschel have tried to make out how much light is needed in order to get a visible curvature. Of course, "visible" is rather vague, but we may express it in such a way that the curvature has to be visible to the naked eye, which is the case when the top is 1–2 mm. out of its original position. Now they were able to show that in order to get this curvature it is necessary to surpass a certain amount of light, which amount can be expressed in meter candle seconds (MCS). That is to say, the intensity of the light in meter candles has to be multiplied by the number of seconds during which the light has been exercising its influence. Hence, it will be clear that this quantity is independent of the intensity of the light. Thus a very strong light has to act only for, say 1/1000 of a second, whereas a feeble light must exercise its influence during several minutes, the product always being about 20. I say about 20, because small deviations from this figure were found as well by Blaauw as by Fröschel. We know now that at least a part of these deviations were a consequence of the imperfect way in which the experiments were carried out. We can do better now and get better results; in the first place we keep the temperature absolutely constant, because it has been shown that the influence of temperature is very great; the same may be said about the degree of humidity of the dark room,

though this is not so important. Secondly, we now use oat seedlings, which are as uniform as is possible, hence we generally use a pure line, a strain of Svalôv. Also, the conditions under which these seedlings germinate are kept as constant as possible; in short, we try to apply physical methods of precision to plant physiology. Taking all these precautions, the result is that in order to get a visible curvature the supply of a certain fixed quantity of light is necessary. And a quantity of light being, physically speaking, an amount of energy, it can be said that a given quantity of light-energy produces a fixed curvature. How far away we are here already from the old conception of a stimulus releasing these movements!

The same thing was found with the sporangiophores of *Phycomyces*, though this was not investigated in such detail as were the oat seedlings. But with *Phycomyces* it could also be shown that the same thing holds true for the negative response, in such a way, that with small quantities of unilateral light a positive curvature ensues; greater quantities result in a negative curvature, whereas yet greater quantities produce a so-called second positive curvature.

Blaauw did not succeed in getting negative curvatures with oat seedlings; this was rather accidental; these curvatures do exist, but they are restricted to a narrowly limited area. Arisz showed this and proved that the matter really is not different as between *Phycomyces* and oat seedlings. This being the case with two so widely unrelated plants, there is some reason to suspect that we have got hold here of a general law in plant physiology. I am not going to delay you by giving a review of all the attempts to find the equivalent law for other plant movements, partly because the time would be much too short for this, and secondly because only with light does the question seem to be tolerably clear, whereas in other cases the notion of what the Germans have called "Reizmenge," "quantity of stimulus," is somewhat hazy, so that of this no well-founded physical basis can be given.

Hence, we will restrict ourselves to light, the more so because there the number of investigations is large enough to get at a theory, which for the moment looks rather sound; we can see our way through here. And a further restriction, now that I have only a limited time at my disposition, is that I shall speak more especially about oat seedlings, except where I explicitly mention some other plant.

We will first of all consider the way in which a curvature appears and its further behavior; this has been worked out in detail by Arisz. In order to see the effect of light, independently of other excitations, it is necessary not only to keep the plants in a dark room after the illumination, but also to eliminate the one-sided action of gravitation. This can be done by a regular rotation around a horizontal axis, for example, by making use of the klinostat of de Bouter. We can adjust this in such a way that regular rotation sets in instantaneously after the one-sided illumination is stopped. Observations are then made with a red lamp, sometimes also a certain stage is fixed by making a photograph of it.

When going on in this way, we see that curvatures always begin at the top and from here proceed slowly to the base, so that more and more basal zones

become curved. But at the same time another movement sets in, which operates to annul the curvature, the concave side beginning to grow faster than the convex one. This is called autotropism, though perhaps autonastism would be a better term; it also begins at the top and proceeds along the same way as the curvature, following the latter after a certain lapse of time. Hence, the top regions may have already straightened at the moment when the base is just beginning to curve; at last the whole seedling is straight again, except the base, where a sharp curvature remains. You will see that it is extremely difficult to measure this curvature; one ought really to consider every zone independently. Up to the present, this has not been possible, and we have to be contented with an approximate measurement, that is, with the deviation of the tip from the original position; we can take this original position to be the vertical line extending through the base of the seedling.

In this way we now can measure the amount of the curvature induced by different quantities of light. Arisz, in doing this, saw that he could go far beneath the threshold of stimulation, which seemed to be fixed by Blaauw and Fröschel. But those investigators took no notice of curvatures invisible to the naked eye, whereas Arisz made use of a magnifying glass, even of a microscope of low power; then the first indication of a beginning curvature may be detected by an inequality of the tip of the seedling. By plotting out the amount of the curvature at different times after the beginning of stimulation we get a line showing a rather steep inclination during the progression of the curvature, but which afterwards becomes horizontal. The beginning of the line cannot be detected, but it looks as if it might almost be continued to the origin of the axes.

If we plot the same lines for different amounts of light, for example, 5, 20, 112, and 600 MCS it will be seen that the greater the quantity of energy administered, the higher the top of the line. These tops, that is, the maximum curvature produced after a certain illumination can be used as a measure of the response. In doing this we get the relation between this value and the amount of the stimulus; Arisz showed that, within certain limits, there is proportionality between these two. It will be evident that there must be a limit here, because on increasing the quantity of light we at last get negative curvatures. On the other side even with 1.4 MCS a curvature ensued; but technical difficulties made it impossible to go further down. So it can only be said that no proof could be given of a real threshold of stimulation, such as was supposed to exist by those who transferred to plants the conceptions of animal, or rather human, physiology.

When we put the question whether it is possible to give an explanation of phototropic movements, we see that two very different theories are maintained. The one asserts that the plant has the power to distinguish the direction of a ray of light. It seems to me that several experiments, more especially by Buder and Guttenberg, have given satisfactory proof of the incorrectness of this view.

The other theory was originally propounded by the older de Candolle. He remarked that stems of plants, grown in the dark, are much longer than those of normal plants, which have developed under periodical illumination; hence, he concludes that light retards the growth of plants. Now in one-sided illumination

the front of the plant gets a greater amount of light than the back, therefore growth here will be more retarded, consequently the plant will bend towards the light. This explanation of de Candolle, though to us it seems to be a little crude, was quite remarkable for the time when it was given. But it could not explain the negative phototropic movements, and also in other respects it was insufficient.

Now Blaauw has again taken up this question, and in order to get an idea of the correctness of this explanation he had first of all to know what is the real influence of light on the growth of plants. In order to study this question he devised a very ingenious disposition of his experiments, measuring growth by means of a horizontal microscope with a red lamp, whereas the illumination was given from four different sides by means of mirrors reflecting the light of a lamp of known intensity during a definite time. He took great care, moreover, to keep the temperature as constant as possible. Now, he found a relation between the quantity of light and growth, so that for a given quantity it is possible to predict what the growth response will be. In most cases the growth reacts with a retardation, which is followed by an acceleration, sometimes afterwards with another similar wave but of much smaller amplitude. With different quantities of light this light-growth response has a different value, and so the front and the back of seedlings which are illuminated from one side do not receive the same amount of light, and must react differently, generally in such a way that the front side is growing slower than the back so that a positive curvature ensues; but sometimes the opposite case occurs and the reaction is negative.

Blaauw made his first experiments with the sporangiophores of *Phycomyces* when the light-growth response consists in an acceleration followed by a retardation of growth. Now Blaauw remarks that the sporangiophores of *Phycomyces* are very transparent, and so unilateral light not only is very little absorbed, but it is broken in such a way that the focus consists of a bright line at the back. That side indeed gets more light than the front, hence grows faster, and in this way a positive response must ensue. Blaauw never did prove that his theory was right, though he made this extremely probable.

Neither did he give the final proof with his seedlings, for which he chose those of the sunflower. It was only his experiments with roots that were rather convincing. He showed that the roots of oats and of radish do not give any response to light, whereas those of white mustard give a very distinct light-growth response. Now, only these last-named roots are phototropic, the others are insensitive to one-sided light.

I pass over several attempts to prove or disprove Blaauw's theory, which had no great success, and will at once speak of Dillewyn's experiments of these last years; they became possible after the construction of a new kind of auxanometer, that is, an instrument for measuring growth. We have seen that Blaauw for this purpose made use of a horizontal microscope and in many cases this is quite sufficient, but the red light, which has to be used here, is always a drawback, more especially so, since Miss Zollikofer has shown that it is not so harmless as was generally supposed. The new auxanometer was devised by Konings-

berger; it is self-registering in such a way that the growing plant can be kept in a room under constant conditions, whereas the registration is carried out in another room, transmitted by means of an electric current. The registration is carried out in such a way that a pen draws lines on a revolving piece of paper; the length of each line gives the time necessary for the growth of 10μ; the longer these lines the slower is growth. With Koningsberger's auxanometer we can get extremely regular registrations. So long as the oat seedlings are kept in the dark, the lines have all the same length, but with the admission of light (which is given here from three sides, making angles of 120°) we get a retardation of growth.

Now, in order to settle Blaauw's theory, van Dillewyn two years ago began experiments about the light-growth response of oat seedlings with quantities of light corresponding to those at the front and back of plants which were illuminated from one side. In order to know with what quantities he had to work, he tried, by means of the blackening of photographic paper, to determine how much light passes through an oat seedling; the same method had been used by Blaauw for other objects. In this way van Dillewyn came to the conclusion that only 1/30 or 1/40 of the light passes through the coleoptile, whereas the other part is absorbed or dispersed. So he had to compare the light-growth response using quantities of light which were in proportion of 1 to 30 or 40; the result was an absolute confirmation of Blaauw's theory.

The best way to see this is to draw graphs for the growth response for both quantities of light on the same abscissa. For these quantities he chose the combination of 2400 MC and 60 MC, the illumination lasting 15 minutes, 90 seconds, and 10 seconds; also a fourth combination of 60 and 2½ MC, lasting 10 seconds. Now in the first two instances the growth line for the larger quantity of light is always below the other one, the stronger illuminated plant grows more slowly than the other one. Hence, if an oat seedling is illuminated from one side with this larger quantity of light, the front will grow slower than the back, so that a positive curvature must be the result. The same may be said of the light quantities of 60 and 2½ MC applied during 10 seconds; also here a positive curvature must ensue when 600 MCS are given one-sidedly. But it is very different with the quantities of 2400 and 60 MC, applied during 10 seconds. Here the latter line lies for the greater part below the other one; hence, it follows that seedlings which have been illuminated during 10 seconds with 2400 MC from one side must show a negative phototropic curvature, preceded by a small positive one. Now every point of these conclusions agrees with the investigations of Arisz, who describes first a positive reaction with small quantities of light (from 1.4–4000 MCS), a second positive reaction with larger quantities of light (from above 100,000 MCS), and between the two—with quantities of 4000–70,000 MCS—a negative reaction often preceded by a positive one.

All this gives a satisfactory confirmation of Blaauw's theory. But we may go farther in the analysis of these phenomena. Darwin was the first to demonstrate that there exists a difference between the behavior of the tip and the

base of grass-seedlings. According to his experiments only the tip would be sensitive to one-sided light, whereas the base would not be sensitive at all, though it always is the principal reacting part of the seedling. Hence, Darwin made the distinction between a perceiving and a reacting zone in the seedling. Now, Rothert, by very careful experiments, showed afterwards that Darwin's conclusions went too far. It is quite true that the tip is the most sensitive part of the seedling, but also the base can respond to one-sided light, though it is far less sensitive than the tip; according to Rothert, sensitiveness would decrease in going from the tip to the base. Only one group of grasses, according to him, was different insofar as they really did behave in the way stated by Darwin; this was the group of the Paniceae. But investigations carried out these last years by Mrs. Bakker-Hazelhoff have shown that there really are no exceptions, even these last behave like the other grasses. Only, the response of the tip passes away very soon, so it is easily overlooked, and the base is so little sensitive that an enormous amount of light is necessary in order to bring about a curvature.

Now the question arose whether these different parts of the seedling do also show a different light-growth response. My son has made investigations in this direction with the result that there is a very striking difference between these two zones. When only the base is illuminated from 3 sides with 500 MCS it shows a very distinct response; almost immediately after illumination a retardation of growth sets in, which reaches its maximum about 16 minutes after the illumination (with a temperature of 25°C.). When only a length of 1.25 mm. of the tip is illuminated with the same quantity of light, the reaction is very different. We get a long response with a minimum of growth after about one hour, and this is followed by an acceleration, which perhaps may be looked upon as an autonomic response of the seedling to the former retardation. Now when we illuminate the whole seedling we get a light-growth response, which is composed of these two reactions; Sierp has made this distinction, so we may speak of a tip response and a different base response.

During these last months van Dillewyn has been making an analysis of the tip, when this alone is illuminated from three sides. His results were such that small quantities of light, for example, 600 MCS, cause a retardation of growth, which becomes less prominent when we augment the light by increasing the duration of the illumination to 6000 MCS. Yet, greater quantities of light, of 60,000 or 600,000 MCS, offer the curious phenomenon of a very distinct acceleration of the growth.

In close agreement were the experiments of Ramaer who followed the line of investigation begun by Boysen-Jensen and Nielsen. They gave a one-sided illumination only to the tip of oat seedlings, after having put a little screen in the middle of this tip, so that only the front received light, and no light could reach the back. Boysen-Jensen and Nielsen, though, made use of continuous illumination, which complicated their experiments. So Ramaer repeated the experiment with definite quantities of light and he obtained the result that with 600 MCS a positive curvature ensued, whereas 60,000 MCS gave a negative response. Now this is in absolute agreement with the just-mentioned experi-

ments of van Dillewyn, who found retardation with 600, acceleration with 60,000 MCS.

Of course the question arises whether it would be possible to get an insight into the cause of the difference between the tip and the base response, the more so because we will probably find a corresponding difference in other cases, such as in the movements of the leaves by means of their stalks, when illuminated laterally, or in some geotropic movements investigated by Jost and Wissmann, or in the movements of some flower stalks. Now, as the base response is a more direct consequence of illumination, it looks as if here we must have some direct influence of light on the living substance of these cells, perhaps not very different from what we see in the pulvinus of the sleeping leaves or of the sensitive plant. We are reminded here of the investigations of Lepeschkin and Tröndle on the influence of light on the permeability of protoplasm. We might suppose that here water is pushed out of the cell sap, just as we can see this in other cases, like the aggregation studied by Darwin and de Vries with the insectivorous plants. But I had better stop these hypothetical considerations, the more so because I shall have to add something to this after mentioning the experiments which have broadened our views about the tip response.

Several years ago Boysen-Jensen, and more especially Paál, procured proof that the tip of a seedling produces substances which regulate growth, either by accelerating, or by retarding it, which substances slowly proceed from the tip to the base. When a tip is cut off, and afterwards is replaced either on the same or on another stump, the influence of these substances on the growth of the base can easily be proved even when a layer of gelatine has been put between the tip and the stump. A sheet of mica, on the other hand, prevents the transmission of this stuff. The way in which the existence of such a substance was generally proved was that of putting the tip laterally on the stump, so that diffusion could only take place on one side of the organ, and to look out for curvatures which might ensue. It is impossible to give in a short time an account of all the work that has been executed in this domain by many investigators, among whom must be mentioned Stark, Nielsen, Miss Purdy, and Miss Seubert, Cholodny and Snow, Beyer and Söding, Brauner, and others. But what I should like to do is to give you some idea of the work carried out in my own laboratory during this last year, because it seems to me that this work perhaps will throw some light on the foundation of all plant movements.

The experiments of van Dillewyn and Ramaer have already been mentioned; I shall only have to say a few words more on these after speaking first of the work of Dolk, who in conformity with Söding showed that after removing the tip of an oat seedling a new physiological tip is regenerated, that is, that one of the functions of the tip, that of producing growth-accelerators, is taken over by the apical zone of the remaining stump. The best way to see this is to fasten an amputated tip laterally on a stump with a little gelatine; then a curvature ensues away from the tip; the base or other parts of the seedling produce no such effect. But $2\frac{1}{2}$ hours after the decapitation the apical part of the stump has the same quality as the former tip, but perhaps to a smaller degree. Here then

proof is given that the sensitiveness for one-sided illumination returns at exactly the same moment as the regeneration of the growth accelerators. So we have to admit that light exerts some influence on existing growth regulators and we have to reject the idea that light would produce specific stimulus-substances.

Several investigators have tried to get a better insight into the nature of these growth regulators. They used the sap, pressed out of the tips of seedlings, sometimes, like Miss Seubert, after mixing it with agar. But the results were negative, or very contradictory. Now my son tried to get at these substances by letting them diffuse out of tips into gelatine; the trial succeeded. He puts tips of oat seedlings a few moments on wet filter paper and after that on a thin layer of 10% gelatine. After about one hour the tips are removed and the gelatine is cut into little square blocks. When these blocks are adjusted laterally on a stump of another oat seedling and fixed with a little gelatine, a curvature becomes visible after about one hour, reaching its greatest size 3 hours after the operation, in such a way that the curvature is always away from the piece of gelatine; we might say that the reaction is negative. Gelatine without such contact with tips never gives this response; only sometimes a slight positive reaction ensues. Nor do we get any result when other parts of the seedlings are placed on the gelatine, except in the case of a regenerated physiological tip, just mentioned, where also the time of regeneration corresponds with the moment when substances begin to diffuse into the gelatine. Hence, the proof has been given that these growth accelerators have diffused into the gelatine.

Provisional experiments did show that desiccation of the gelatine does not destroy these substances; neither are they destroyed by heat (even heating during 1 minute at 90° C. had no effect). By mixing this gelatine with some of the same stuff into which no diffusion had taken place, it became possible to attenuate the action on the stumps even quantitatively.

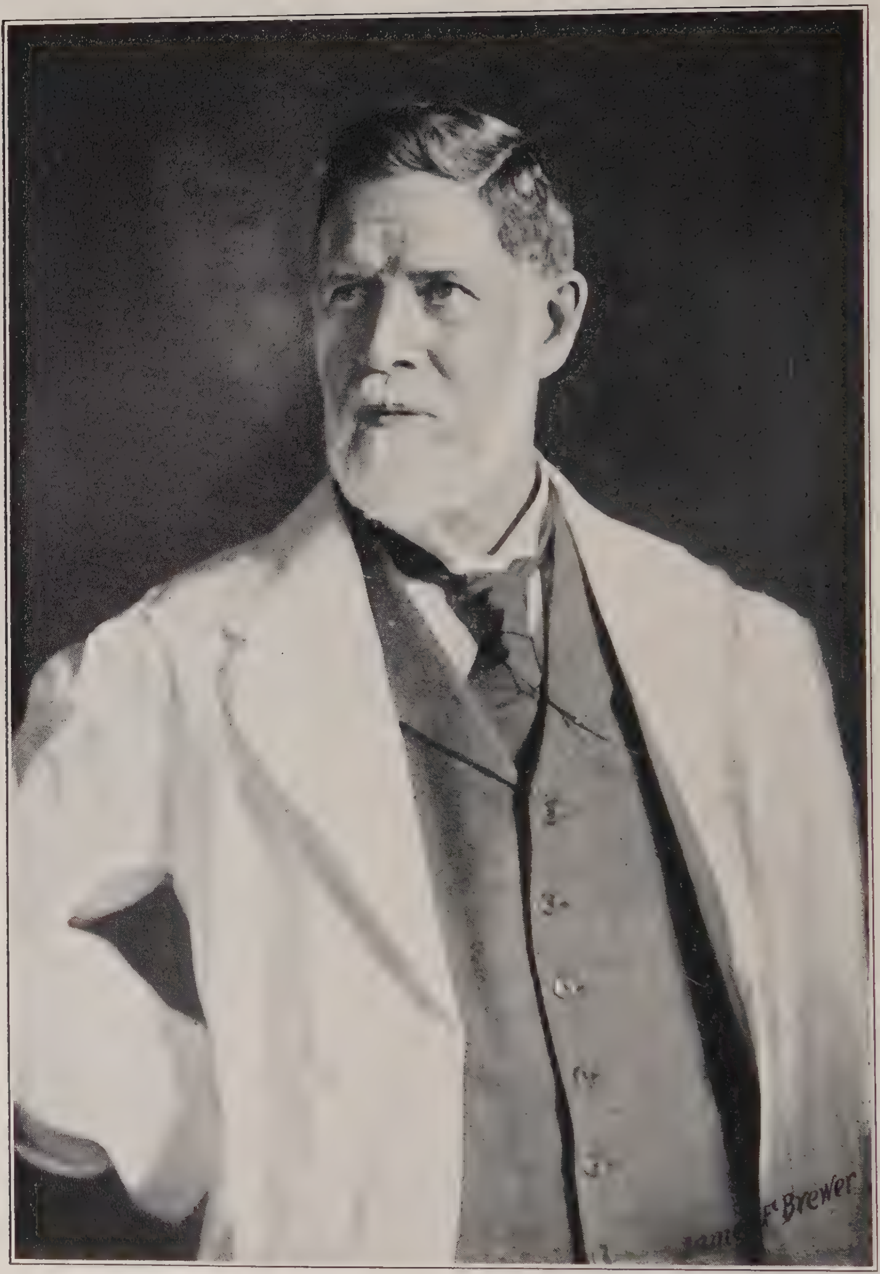
Preliminary studies gave the result that light has no action on these regulators as soon as they have diffused into the gelatine. On the other hand, it was evident that tips of illuminated plants must behave differently from those which have been kept in the dark; this indeed was the case. It was proved that tips illuminated with 1000 MCS produced less growth regulators than those which had been kept in the dark, and on the other hand, illumination with 100,000 MCS or more increased the quantity of these substances. This is absolutely in agreement with the results of the investigations of van Dillewyn and Ramaer, just mentioned. It now became possible also to imitate the phototropic response by putting on a stump two blocks of gelatine into which growth regulators had diffused which originated from tips submitted to two different illuminations. I cannot enter into the details, but must mention that here again the results were in perfect agreement with those of the other investigators.

One point more must be mentioned briefly. Now that we have these blocks of gelatine with growth regulators, we can substitute this physical system for the natural tip. We are then able to investigate the influence of different outward factors on the base, with the presence of growth regulators which themselves are not submitted to these factors. Time does not permit me to do more than

give a small hint here. It suffices to say that my son proved that light retards the velocity of transmission of these growth regulators to the base. How does this transmission go on? Though the light-growth response of the tip does not begin as immediately as the base response, and though this time of latency certainly is due to the transmission of the growth regulators to the base, yet diffusion is a process much too slow to offer any explanation. We will have to resort to the living protoplasm, as Bose is doing in the case of the transmission of the stimulus in *Mimosa*. I think that there is some probability of protoplasmic streaming playing a part in it; thus we would be taking up an old hypothesis of de Vries, which until now, though, has not found much recognition among botanists.

It was a long way which led us from anthropocentric conceptions of plant movements to our present knowledge. We do not assert that this knowledge is very great, only we believe that there is but one way which will lead us further, and that is by experiments on an exact scale, as exact as possible; I should say almost as exact as the experiments of physicists. We certainly will not get on by philosophizing and building up great systems, making use of terms and conceptions, borrowed from human physiology or even psychology. I also loathe the use of words like hormone in plant physiology; when we say growth regulator, we say nothing more than we can stand for, whereas the word hormone has a certain meaning in animal physiology which would have to be altered, in order to make use of it for designating the growth regulating substances of which we have been speaking.

And to me it is evident that the moment botanists began their investigations without the handicap of all sorts of cloudy terms, the mist began to clear up, so that some day we may hope to get a better insight into these phenomena. Instead of going along the old beaten track, it looks as if we might get a much better and larger view by taking a new road. Once we have succeeded in penetrating somewhat further into these plant phenomena, that certainly will give us a better insight also into the much more complicated stimuli of animals. Of course, animal physiology cannot wait till we have gone so far, but I think that, perhaps to take a little more notice of the results of those who study the vital phenomena of plants, than is now bestowed upon such results, would be of some value for the human and the animal physiologist.



ERWIN F. SMITH
1854–1927
SMITH: FIFTY YEARS OF PATHOLOGY

FIFTY YEARS OF PATHOLOGY[1]

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In many ways the last 50 years is a remarkable period in the history of the world. Among its striking characteristics, the most impressive perhaps have been the enormous advances in scientific discovery. This period has seen an entire change in our views as to the constitution of matter, the development of living things, and the extent of the cosmos. Outward in various directions the visible universe has been extended thousands of light years, downward it has been extended into the minutest subdivisions of matter far beyond the "atoms" of the old Greeks, or the wildest dreams of Victorian physicists and chemists. In pathology the advances have been no less wonderful.

[1] Invitation address before the International Congress of Plant Sciences, session of the Congress as a whole, Ithaca, New York, Aug. 20, 1926.

[*Editorial note.* The completion of this paper was one of the significant contributions with which Erwin F. Smith was engaged at the time of his death, April 6, 1927. It is a dramatic coincidence that practically at the close of his devoted review, "Fifty years of pathology," he should himself be removed from participation in the new period, and thus the old which he described is more emphatically closed.

It is not the editor's privilege to add to what others have done in reviewing this life, so well filled, as it was, with keen artistic appreciation, with ever-helpful human sympathy, and with ncreasing scientific accomplishment. My duty is merely to announce to the readers of the Proceedings that this paper is presented essentially as it was read at Ithaca. In fact, one may infer that he paper was written primarily to be read and that it was not closed as a complete record of the events which Doctor Smith wished to emphasize. One is also conscious of the fact that he was dominated by a sense that he must compress the work, particularly that of the last ten or twenty years.

That emendations and additions were contemplated is perhaps best evidenced by the finding of two copies of the paper, each with marginal notes peculiar to the copy. But to preserve the full spirit and dominant interests of the author, the editor and those with whom he has consulted were definitely agreed that only such slight changes as were obviously necessary should be introduced Completing the preparation of the plates was a matter which could not be sympathetically carried out by anyone not in the closest touch with Dr. Smith during some of the time he was engaged in collecting the portraits. Fortunately, it was assigned to Mr. James F. Brewer, long associated with Dr. Smith, to complete the undertaking, and it is to Mr. Brewer that much credit is due for carrying through this phase of the work—guided by such notes as the author left, and with a cordial appreciation of the spirit of the endeavor.

I am assured that during those last days of his life Dr. Smith derived great pleasure from the pictures which continued to come, each with a kindly or complimentary greeting. From the extent of his requests and the evidences of his plans, it would seem that he intended to maintain and develop the portrait gallery, perhaps with supplementary publications at later times. *Editor.*]

I shall endeavor to discuss this subject broadly in its main features only, and I cannot do so for plants without paying some attention to the diseases of animals and of man, for the subject is really one subject with the same general principles of procedure in research and the same underlying laws. Moreover, plant pathology is tremendously indebted to animal pathology in many ways.

In the limited compass of a single address it is possible, of course, to touch only on the high-lights of this great subject. Its full treatment would require a thousand pages and the mention of a thousand names.

Three main currents of thought and pathological influence flowed into this 50–year period from earlier times. These three streams were (1) the purely systematic mycological influence of such men as Corda, Elias Fries, and the Tulasne brothers; (2) the experimental and observational work on the morphology and etiology of human diseases by Henle, Cohnheim, and Virchow and of plant diseases by such masters as Farlow, Berkeley, Kühn, and De Bary; (3) the bacteriological and animal pathological researches of Louis Pasteur and his school. The last influence was greatest of all, but each current interacted with the other two, and all of them on the men of this period.

Fifty years takes us back to 1876, a memorable year in various ways. In the United States it was the year of the Centennial Exposition in Philadelphia and the year that Alexander Graham Bell in Boston sent his first message over the telephone. Asa Gray, John Torrey, and George Engelmann were actively studying our Flora. W. G. Farlow, of Harvard, recently back from De Bary's Laboratory, was working on onion smut. C. V. Riley was publishing in Missouri on injurious insects and on insecticides. T. J. Burrill, in Illinois, was studying fungi and had just taken up pear blight, the bacterial cause of which he was to announce a few years later. Paris green was coming into general use as an insecticide for the Colorado potato beetle which had now made its way to the Atlantic seaboard.

In England, Berkeley, Cooke, and Plowright were studying and publishing on fungi. Worthington G. Smith, backed by the "Gardeners' Chronicle," was squabbling with De Bary over the morphology of the potato fungus, *Phytophthora infestans*. Bentham and Hooker were continuing their great "Genera Plantarum," and Darwin had just published his "Variation of Plants and Animals under Domestication."

In France Louis Pasteur had finished his studies of the silkworm diseases and was laying the foundations of bacteriotherapy by his studies of anthrax and the septic vibrio. His great triumphs in vaccination for chicken cholera, anthrax, and rabies were to come a few years later.

In Strassbourg Anton De Bary was studying critically many kinds of fungi, including plant parasites, and his successful infections on plants, equally with those of Pasteur on animals, led many medical men to wonder if human diseases might not be due to parasites. De Bary was also attracting to his laboratory students from all over the world, many of whom were to become famous later on. Ferdinand Cohn, in Breslau, was publishing his "Beiträge," and Robert Koch in Cohn's Laboratory, was beginning his phenomenal career by demonstrating

the spores of the anthrax organism. Julius Sachs in Würzburg, was doing beautiful research on the physiology of plants, and Julius Kühn, also in Germany, was working on plant diseases, while Oskar Brefeld was studying the life history of Basidiomycetes.

It requires a serious effort to put ourselves back fifty years and think as people then thought. Indeed, it is an almost impossible feat for one who has not lived through the period. I may not be able to carry you along with me, but I shall try. There are the lantern slides for those who cannot hear me. Fifty years ago there were no systematic works of any consequence on the smaller plant and animal parasites. Very little was known as to the cause of human or animal diseases or as to remedies for such diseases. All the journals and manuals of bacteriology and of animal pathology were things of the future. Even as regards most plant diseases we were groping about in the dark. The activities of certain parasites were very deadly, medication was exceedingly primitive, and those of us who desired to study these things experienced the greatest difficulty in finding teachers and books. Truly it was a time of groping about! But the darkness was about to disappear, indeed it had disappeared from the minds of a few great men like De Bary, Pasteur, and Koch, but they had active opponents and nobody knew where the truth might be.

I shall have to go a little into detail to make my meaning entirely plain. Fifty years ago France was in the throes of desperation and her vine growers were bankrupt from destruction of her vineyards by *Phylloxera*, and there were no remedies. All north Europe was ravaged year after year, beginning about 1845, by the potato mildew, which caused a famine in Ireland, and there was no remedy. Pear blight was more or less destructive every year in the great apple and pear orchards of the eastern United States, and there was no remedy, nor was the cause known. The splendid coffee plantations of Ceylon were being destroyed wholesale by a rust fungus, and there was no remedy. Typhus fever, typhoid fever, diphtheria, tuberculosis, syphilis, cholera, yellow fever, and the plague destroyed hundreds of thousands of persons every year, and the causes were not known and there were no remedies. There was a shot-gun quarantine and disinfection of clothing (now known to be useless) for yellow fever, and with stenches in the houses there were prayers in the churches against yellow fever, cholera, and other epidemics. Malarial fever destroyed or invalided multitudes of persons every year and rendered a large part of the tropics uninhabitable to the white man, and nothing was known as to its cause or as to how it was contracted. Many supposed it was due to breathing night air. Following Pettenkofer, the cause of typhoid fever was supposed to be a miasm in some way related to the depth of the ground water. No one suspected that deadly diseases could be transmitted by flies, mosquitoes, ticks, bedbugs, fleas, lice, cats, rats, rabbits, goats, antelopes, and other animals, or that an apparently healthy human being (one recovered from a disease) could remain the carrier of its germs, deadly to another. Such ideas were all on the knees of the gods. The world was not ready for them. It was too much absorbed in money-making, pleasure, politics, war, and other-world religion to pay much attention to causes of disease, nor

was the scientific man properly equipped for the study of minute parasites. Apochromatic glass and cultural technique were things of the future.

Fifty years ago there was no science of bacteriology, no immunology, no cytology, no genetics, no plant or animal pathology worthy of the name, no public health service founded on exact knowledge, and in nearly every case no well-ascertained remedies. In fact, there was no medicine such as we now have based on the circle of the sciences, but only squabbling schools of empiricists called Homeopaths, Allopaths, Hydropaths, and Eclectics. Osteopaths and Christian Scientists had not then emerged above the horizon. We had vaccination for smallpox, various laxatives and sudorifics and cold baths for fevers, anesthetics and opium to dull pain, digitalis for weak hearts, quinine for malaria, and mercury for syphilis and various intestinal diseases. Many other drugs, of course, we had, but taking them was like shooting at desperadoes in the dark, or like throwing sand into delicate mechanisms, and surgery was notoriously deadly. Surgeons did not venture to enter the thoracic cavity or the abdominal cavity, or if they did their patients died. Our knowledge of germicides, fungicides, and insecticides was of the crudest. We had sulphur for grape *Oidium*, carbon bisulphide and long-flooding for *Phylloxera*, and arsenites for gnawing insects. There were no journals devoted to plant or animal pathology.

What a marvelous change we have seen! In these matters the scientific discoveries and applications of the last fifty years have been worth more to the physical well-being of plants and animals and man than all the discoveries that preceded them back to the days of Aristotle and Æsculapius.

Fifty years ago appears to be a very benighted time when we look back upon it from our present daylight, but it did not seem so to us when we were in the midst of it. We were young then, and full of hope, and a hundred enticing paths opened out before us in every direction, beckoning us on.

I wonder if the young men and women who are listening to me will consider this also as one of the dark ages, fifty years from now. Very likely, for, if we are not plunged again into medieval ignorance, through fundamentalism, or communism, or war, we may expect the advance of science to be simply tremendous in the near future, opening up arcana in every direction.

Let us turn now and trace some of the lines of advance since 1876.

1877–1886

There were giants in those days. For a decade Pasteur had sown the good seed of the new ideas and it was germinating and blossoming in every direction. The first decade of our 50 years leads all the rest. The good Woronin had just explained the nature of club-root in turnips and cabbages (1876), and a great deal was already known about various fungi and their habits, but not much about the bacteria. There was no good technique of bacteriology in those days, you will remember, and in every direction Egyptian darkness prevailed. Many deep-seated prejudices and misconceptions of long standing had to be overcome. Bacteria had been seen previously in some of the diseases I am to mention but they were thought to be concomitants or followers rather than causes of disease.

The scientific men of that generation who concerned themselves with problems of etiology were obsessed with the idea that disease, especially in man, was a degeneration, or due to a miasm (a gas or vapor) or to some chemical decomposition arising within the body for some unknown reason, and the common people regarded disease as an awful visitation of God, as they still do to some extent.

In the middle of this decade (1878–1880–1883), using the microscope, Burrill demonstrated bacteria to be the only organisms constantly present in fire-blight of the apple, pear, and quince, and with diseased material full of these bacteria he reproduced the disease in healthy trees, by inoculation, but it cannot be said that he convinced the public. Old ideas die hard. Pear blight was thought to be due to insects, to lightning, to sour sap, to frozen sap, etc. Arthur, following Burrill, and after the latter had ceased to work on the disease, demonstrated, to further convince a sceptical generation, that the bacteria when passed through six fluid cultures in series would still produce the blight. Arthur also separated the bacteria from the fluid surrounding them by means of clay filters and proved that the liquid would not produce the disease, whereas the bacterial residue on the filter was still actively pathogenic.

By the year 1877 the Colorado potato beetle had overrun the whole eastern United States, and C. V. Riley, then state entomologist of Missouri, said: "By means of intelligence and a little paris green the American farmer is pretty much master of the *Doryphora*."

In 1879 the hollyhock rust was bad in England and Germany. That year De Bary became sole editor of the "Botanische Zeitung."

In 1877 Bollinger produced lumpy jaw of cattle (Actinomycosis), by means of the ray-fungus. In this period, which is the Burrill pear-blight period (1877–1882), Pasteur was writing on anthrax. He had discovered that the spores were distributed by earthworms and had found a remedy in a vaccine made from an attenuated culture. In 1877, 1878, 1880, Pasteur was also writing and speaking on wound infections and the cause of puerperal or child-bed fever and on the necessity of an entire change in surgical procedure in order to avoid the frightfully prevalent hospital infections.[2] In 1880 Pasteur and his associates demonstrated bacteria to be the cause of chicken cholera and of boils, discovered a preventive of the chicken disease in an attenuated virus, and laid the foundations of immunology. In 1882 Pasteur and his associates discovered the cause of measles in swine and, greatest triumph of all, found a remedy for hydrophobia in an attenuated virus, without, however, discovering the cause of the disease. Last year in Paris I talked with Joseph Meister the first person ever vaccinated for this disease (1885), then a boy of 9.

[2] In this warfare he had two predecessors: Dr. Oliver Wendell Holmes, Boston, 1843, and Dr. Ignaz Semmelweis, in Vienna, 1847–9 and again in Budapest in 1861, both of them were right, but failed to convince an unwilling public because neither had Pasteur's technique or his aggressiveness. Of Semmelweis who was treated very badly by some of his colleagues and who became insane, Dr. Garrison says: "He is one of Medicine's martyrs and, in the future, will be one of its far-shining names, for every child-bearing woman owes something to him" (History of Medicine).

In 1879–1880 Arloing, Cornevin, and Thomas discovered the cause of the symptomatic anthrax of cattle.

In 1879 Neisser discovered the gonococcus, cause of gonorrhoea, of one form of ophthalmia leading to blindness, of epidemic vulvovaginitis in children, and of other serious troubles.

In 1878 Robert Koch discovered the anthrax *spore* which served to explain many things until then very obscure. In 1882 he invented the poured-plate method of isolating bacteria on solid media. At first we had long strips of glass, exposed to the air, on which strips of gelatin were poured. This led to humorously incredulous remarks by Virchow, that every organism would need to have a culture chamber of its own before there could be any certainty, something we actually did have a little later in the convenient Petri dishes which we still use in enormous numbers all over the world. This method of Koch was a great advance on the dilution method of Pasteur and one which led to immediate practical results in every direction. No single discovery has contributed more to the advancement of bacteriology, and along with it, pathology, than this simple method, unless it be the equally simple cotton plugs to protect test-tube and flask cultures from the bacteria and fungi of the air.

In 1881 Robert Koch published his important paper on wound-infections. In 1882 he demonstrated in laboratory animals that tuberculosis could be produced by inoculating pure cultures of a microorganism cultivated from tubercles. In 1883 he announced his discovery of the cause of Asiatic cholera, followed, the next year by a long paper on the same subject, with all necessary proofs. In 1884 Koch also published his magnificent long paper on the cause of tuberculosis, a model for all time. What a year that was! and what enthusiasm these two papers evoked! I remember it all as if it were yesterday, and much of the old thrill comes back! I knew very little about Pasteur in those days, but Robert Koch was my hero, and his influence more than that of any other man decided me to enter pathology and bacteriology. We are not done yet with that marvelous year 1884, as we shall see a little later.

In 1880 Armaner Hansen, in Norway, discovered the bacillus of leprosy, and in 1882 Loeffler and Schütz discovered the bacillus of glanders.

In 1881 Tyndall wrote on floating matter in the air in relation to putrefaction and infection, and he it was who recommended discontinuous sterilization, another superb simple procedure.

In 1882–1884 Lavaran published on the protozoan parasites of malarial fever in man, first seen by him in 1880 in the red blood corpuscles.

In 1883 Eberth discovered the bacillus of typhoid fever, and during the next two years Gaffky verified Eberth's findings and cultivated the bacillus from human spleens.

In 1883–4 Friedländer discovered the *Pneumococcus*. In 1882–1884 Willoughby D. Miller, an American dentist living in Berlin, associated bacteria with dental caries, and subsequently called repeated attention to the danger of systemic infection from neglected teeth. In 1881, 1882, 1883 Fehleisen wrote on a *Streptococcus*, the cause of erysipelas.

In 1884 Watson Cheyne discovered *Bacillus alvei* in foul brood of bees. The same year (1884) Loeffler discovered the cause of diphtheria, and Nicolaer the cause of tetanus That year also Chauveau and Arloing wrote on septicaemias, and the following year (1885) Hesse published on malignant oedema.

In 1884 Marchiafava and Celli saw the meningicoccus of meningitis and correctly described its location, but Weichselbaum was the first to isolate it in pure culture (1887). Marchiafava and Celli subsequently differentiated the parasite of tertian malaria, and Celli wrote much on the occurrence and prevention of malaria in Italy.

Coming over again to the plant side, in 1879 Cunningham described *Mycoidea parasitica*, a tropical alga occurring in the leaves of various plants. The same year in Europe Kühn discovered *Phyllosiphon arisari*, another alga, parasitic in the leaves of *Arisarum vulgare*. In 1880 Marshall Ward went to Ceylon to study the coffee disease where the total losses from that disease in the 10 years (1869-1878) were estimated at 15 million pounds sterling. That year the "Botanisches Centralblatt" was founded. In 1882 Darwin died, and Saccardo began the publication of his great "Sylloge Fungorum," destined to be a summary of everything known about fungi. In England Plowright was describing diseases due to rust fungi. M. C. Cooke and Worthington G. Smith were also writing, and the latter was making innumerable handsome wood-cuts, not always strictly accurate, that is, sometimes he attached his spores wrong end to, and often he jumped at conclusions.

In 1882 appeared the first edition of Robert Hartig's "Lehrbuch der Baumkrankheiten."

In 1883 Wakker, in Holland, published his first paper on the yellow disease of hyacinths.

In 1883 the "Berichte d.d. botanischen Gesellschaft" was begun, a mine of information on botanical subjects.

In 1884 De Bary published his magnificent "Vergleichende Morphologie und Biologie der Pilze, Mycetozoen, und Bacterien" which was our *vade mecum*, albeit the German was rather tough reading. I find a note in my German copy (we had no English translation then): "Begun July 7, 1886. I must master all the details of this book;" and the first 200 pages are pretty well marked up. Then came the Garnsey-Balfour translation (1887).

In 1886 Farlow published his third paper on the Gymnosporangia of the United States.

In 1885 Millardet recommended a mixture of sulphate of copper and quicklime for grape mildew, and within the next few years it was demonstrated everywhere to be a sovereign remedy not only for the ravages of the grape *Peronospora*, but also for many other diseases of cultivated plants, including black rot of the grape and the devastating mildew (*Phytophthora*) of the potato. This was the first great advance in plant therapeutics. The formula then given was 18 pounds of copper sulphate dissolved in 22 gallons of water and 34 pounds of stone lime slaked in $6\frac{1}{2}$ gallons of water. The 2 liquids were to be mixed and

sprinkled on with a broom. It was still a primitive procedure. That year (1885) the section of mycology was established in the United States Department of Agriculture with F. L. Scribner as chief, and the following year (1886) the writer was made his first assistant, and both of us were absorbed in the treatment of grape diseases and in getting the new French ideas on copper fungicides into English for the use of grape growers along the Atlantic Seaboard where mildew and black rot prevailed.

In 1886 Adolf Mayer in Holland showed that tobacco mosaic was a communicable disease later shown to be due to a filterable virus. The first one discovered if I am not mistaken.

In 1886, von Thümen, the Austrian, published his little book on the control (Bekämpfung) of fungous diseases of cultivated plants, concerning which the "Gardeners' Chronicle" in a kindly vein remarked: "In the present state of plant pathology, an attempt to write a book on plant therapeutics is as bold as the results are certainly modest."

In 1886 also appeared the first and second volumes of the greatly improved second edition of Sorauer's "Pflanzenkrankheiten," a godsend to all of us who could read German. We had at this time in English very few serviceable books. There were not many papers written on plant diseases at that time, nor had we any journals devoted to that subject in any language.

In English there were few books of any sort, good or bad. I recall Berkeley's "Outlines," with a chapter on plant diseases which was good but old (1869) and very incomplete, and Worthington G. Smith's "Diseases of Field and Garden Crops," newer (1884) and more extensive but less dependable. There was also a little book on "Rusts, Smuts, Mildews, and Moulds," by M. C. Cooke (1865), which did service of a sort in default of something really good. I used also a little German book by George Winter (1878). We had to be content with books and papers in Latin, French, and German, and largely with systematic treatises on fungi. So we read Berkeley and Tulasne, Corda and Fries, Montagne and De Bary, and all the older writers, and English and American plant pathology in this decade was little more than a sublimated mycology.

1887–1896

The second decade was a period of readjustment and of taking stock of all the new discoveries, but it was also a period of great research activity and there were distinct advances in several directions.

On the plant side, copper fungicides were varied in many ways in all the countries, and tested on a great variety of plant diseases. In the United States, Galloway of the federal Department of Agriculture (1889) was one of the leading spirits, and under him the section of mycology became the Division of Vegetable Pathology (1890) and branched out in various directions, becoming a leading influence for the advancement of plant pathology in the United States. In England, Plowright and Marshall Ward became strong men. In Holland, Beyerinck and Ritzema Bos; in Denmark, Rostrup; in Germany, Hartig,

Frank, Sorauer, Brefeld; in Italy, Comes, Cuboni, Cavara, and Savastano; in France, Viala, Ravaz, Millardet, G. Foëx, and Prillieux all exerted a marked influence on the progress of plant pathology.

In 1887 Engler and Prantl commenced their vast publication "Die Natürlichen Pflanzenfamilien," of service to everyone.

In 1888 Woronin published his beautiful monograph on Sclerotinial diseases of Ericaceae.

In 1889 Plowright published his monograph on the British Uredineae and Ustilagineae, and throughout this period contributed numerous notes to the "Gardeners' Chronicle" on plant diseases. Worthington G. Smith also wrote much on plant diseases during this period for the same journal.

Pierre Viala, who made a special treatise on grape diseases in 1885, came to the United States in 1887 to study them from one end of the country to the other. In 1888 Viala and Ferrouillat made a manual on the treatment of vine diseases. Various sorts of apparatus for spraying and dusting were figured and the chief dependence then was on sulphur compounds and copper compounds, just as it is today. Plant therapy has not advanced very rapidly in the last 40 years.

In 1888 appeared Farlow and Seymour's "Host Index of the Fungi of the United States." The same year Marshall Ward described a *Botrytis* disease of lilies.

In 1888 De Bary died. In 1889 Berkeley died, and of him the "Gardeners' Chronicle" said: "Mr. Berkeley was the first, and till quite recently, the only botanist in this country who devoted systematic attention to the diseases of plants."

In 1889 Kellerman and Swingle were describing Kansas fungi and recommending hot-water treatment for smut in wheat.

In 1889–90 Roland Thaxter described the downy mildew of lima beans, and in 1890–91 he discovered and described the cause of potato scab.

In 1890 Byron D. Halsted wrote on various sweet-potato diseases in New Jersey.

In 1890 Galloway and Fairchild were saving 93 to 99 per cent of the grape crop from black rot in Vienna, Virginia, by means of copper fungicides at a cost of 1 to 2 cents per pound of fruit, the untreated vines yielding nothing.

In 1890 Southworth and in 1891 Atkinson published, independently, on anthracnose of cotton; Halsted and Fairchild published on the black rot of the sweet potato; and the writer described blossom blight and twig blight of the peach, due to *Monilia*.

Beginning with 1891, every year, Halsted published from the New Jersey Experiment Station illustrated reports on diseases of plants.

In 1892 Iwanowski, the Russian, showed that the virus of tobacco mosaic could be passed through a Chamberland filter without losing its power to infect.

In 1893 B. T. Galloway and in 1896 L. R. Jones published on the *Macrosporium* disease of potatoes.

In 1890 Scribner published his "Fungus Diseases of the Grape and Other Plants and their Treatment" (J. T. Lovett Co., Little Silver, N. J., pp. 136, portrait and 64 figs. in the text from his pen drawings). This was the first plant pathology published in the United States.

In 1890 Newton B. Pierce went to France, Italy, and North Africa to study vine diseases for our United States Department of Agriculture. About this time, in California he demonstrated that peach leaf curl could be controlled by fungicides, the publication of his data being much delayed, meanwhile B. M. Duggar demonstrated the same thing in the East.

This was the period in which the mysterious Anaheim vine disease prevailed disastrously in California (1887–1889) and in which the writer studied the equally destructive yellows of the peach in Maryland, Delaware, and Michigan; and discovered (and transmitted by budding) rosette of the peach in Georgia; two diseases which are still enigmas.

This was the decade in which Wakker and Went studied a variety of sugar-cane diseases in Java (1893–1896) and published many interesting papers.

Much additional information concerning the morphology, cultural characters, and pathogenic properties of the pear-blight organism, left uncertain by Burrill and Arthur, were obtained through the studies of Merton B. Waite, and several new plant diseases were ascribed to bacteria, 5 correctly. These were the olive tubercle (Savastano, 1887), the soft rot of hyacinth (Heinz, 1889), the wilt of cucurbits (Smith, 1893), the bacteriosis of rutabaga (Pammel, 1895), and brown rot of tomato, potato, and eggplant (Smith, 1896).

It was in this decade (1894) that the writer began his studies of parasitic Fusariums, finding them in cotton, melons, cowpeas, and cabbages, and called special attention to the fact that this form-genus, hitherto generally supposed to be saprophytic, contained a number of very destructive soil parasites.

In 1892 Pierce in California, by hybridization, obtained grapes resistant to the Anaheim disease.

In 1893 Victor Peglion in Italy, discovered a grave *Alternaria* disease of muskmelons and the following year, without knowing of Peglion's work, the writer observed it destroying whole fields of melons in southwestern Michigan, isolated the fungus in pure culture, and established its pathogenicity. This was the beginning of our friendship. He has since written a plant pathology, and is now Minister of Agriculture in Rome.

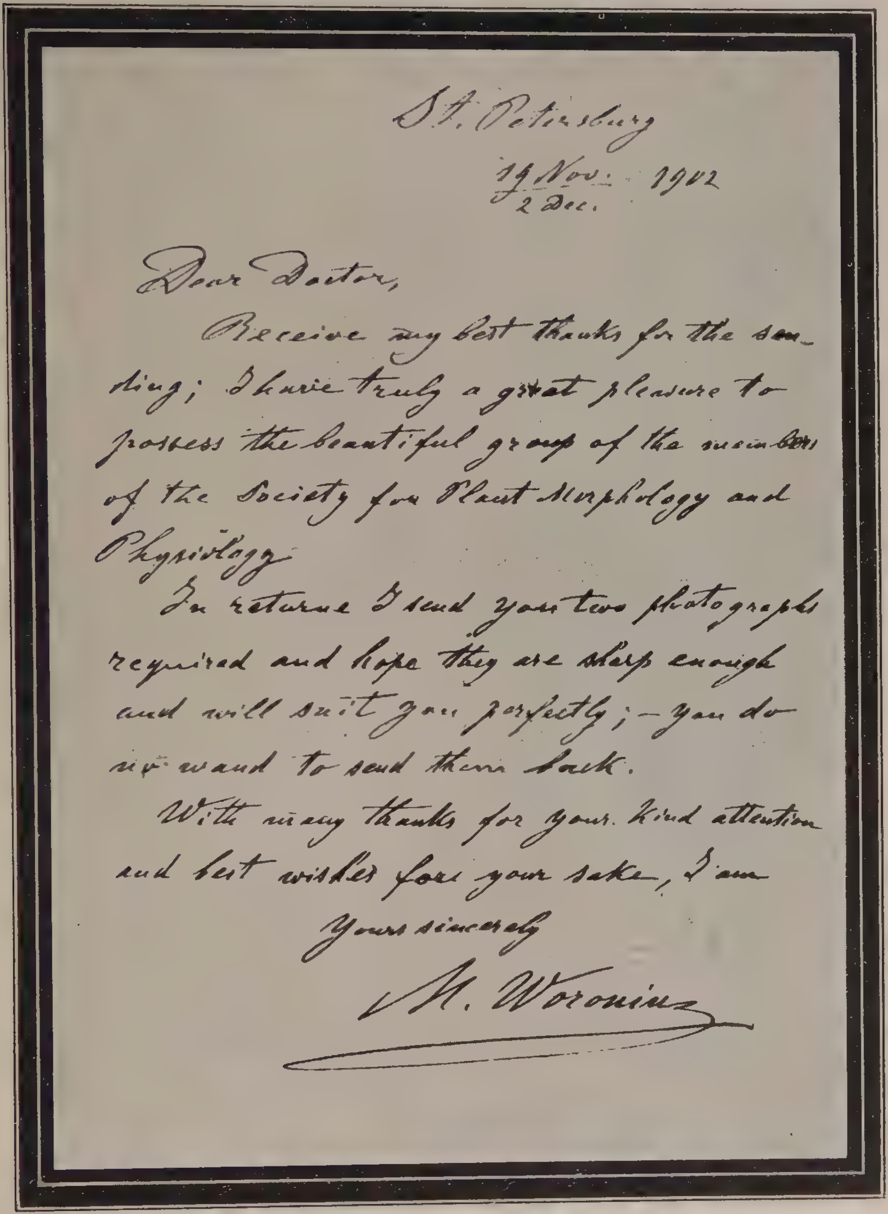
In 1894 Atkinson contributed to our knowledge of the leaf curl fungi (Exoascaceae) of the stone fruits.

In 1895 Woronin published his superb monograph on Sclerotinias attacking *Prunus* and *Sorbus*. For one of his letters see the text figure.

In 1895 or perhaps earlier, Hermann von Schrenk began his study of the fungus diseases of forest trees in the United States, now a large field with many workers and a comprehensive literature, but German work on forest pathology was earlier than that of the United States.

Useful books and papers on plant diseases multiplied. Kirchner (1890), Comes (1891), Tubeuf (1895), and Prillieux (1895) all published hand-books

on plant diseases. In 1893 appeared the third edition of Viala's "Les Maladies de la Vigne" (600 octavo pages, fully illustrated).


St. Petersburg
19 Nov. / 2 Dec. 1902

Dear Doctor,

Receive my best thanks for the sending; I have truly a great pleasure to possess the beautiful group of the members of the Society for Plant Morphology and Physiology.

In returne I send your two photographs required and hope they are sharp enough and will suit you perfectly; — you do not want to send them back.

With many thanks for your kind attention and best wishes for your sake, I am

Yours sincerely

M. Woronin

A Letter From Woronin to Erwin F. Smith (Woronin died the following year.)

The "Annals of Botany" was begun in 1887, the "Journal of Mycology" under Government auspices in 1889, Sorauer's "Zeitschrift für Pflanzenkrank-

heiten" in 1891, the "Revista di Patologia Vegetale" in 1892, and the 2te Abteilung of the "Centralblatt für Bakteriologie" in 1895. Many of the older botanical journals also began to carry notes or papers on plant diseases.

In 1893 the "Botanische Zeitung" (De Bary's journal) was 50 years old.

During this decade *Phylloxera* was definitely overcome in the French vineyards by flooding, by insecticides, and especially by grafting *Vitis vinifera* on resistant American vine-stocks. This disease, introduced from the United States, ravaged the vineyards of France for more than 20 years. The yield of wines, according to Viala, fell off from 83,632,000 hectoliters in 1875 to 23,000,000 in 1889, while about 1,500,000 hectares of vines (more than 3 and 7/10 million acres) were entirely destroyed, that is, three-fifths of the whole vine industry of France. I have no records at hand for Germany, Austria, and Italy, but *Phylloxera* also spread into and ravaged the vineyards of these countries.

Speaking of the introduction of parasites, all of the more destructive vine diseases of France were introduced on vines brought from the United States, and this illustrates how dangerous it is to introduce plants bearing parasites into new environments suited to the parasites and free from their enemies, especially when they may escape to more sensitive plants, as we shall see by fresh examples when I come to deal with recent introductions into the United States.

During this period, the San José scale, introduced into California from eastern Asia and confined to the Pacific Coast for a considerable period, was distributed all over the eastern United States by a couple of unscrupulous New Jersey firms, on infested nursery stock, of which they had a great quantity, and here it became a great peril in orchards toward the end of this second decade. A partial remedy was found in a lime-sulphur spray, but as this defoliated the trees it had to be applied in the winter season, and the amount of energy required to control the scale was so great that only the more active orchardists succeeded. All the others lost their trees.

Under Coquillett's direction in California hydrocyanic acid gas was used early against the San José scale and very effectively under tents in orange orchards, and it has been used since extensively everywhere for the fumigation of dormant nursery stock, and in light doses on growing plants in hothouses for various aphides and scales.

In 1896 Bolley recommended treatment of seed tubers with corrosive sublimate as a remedy for potato scab, and the same year Chester recommended sulphur for this disease.

In this decade tea culture was substituted for coffee culture in the plantations of Ceylon, owing to the ravages of the rust fungus *Hemileia vastatrix*.

The greatest advance of this decade, probably, was the discovery that diseases of animals and plants may be disseminated by insects and arachnids. This idea did not come wholly out of the blue. Nothing ever does. Various persons had ascribed Texas fever to cattle ticks. Arthur had suspected insects of carrying pear blight and Carlos Findlay, of Havana, as early as 1881 had suspected the mosquito of being the transmitter of yellow fever, and had actually

pointed out the particular one that does it (*Aëdes aegypti*). But these were happy guesses unlike a thousand others. There have been innumerable hypotheses on all sorts of subjects, some very clever ones, from the time of the Greeks, but we must carefully distinguish between hypothesis and proof.

What Arthur and Findlay and others suspected detracts in no way from the honor due to the men who first experimentally demonstrated the existence of carriers of disease; nevertheless honor goes also to the men whose theories in the hands of other men come true. So many men have builded on this early work and it has become of such great practical importance in the prevention of disease that the beginnings are unusually interesting. These all belong to the credit of the United States Department of Agriculture. There Merton B. Waite, working on pear blight, proved conclusively the transmission of the disease by bees, from whose mouth parts he again cultivated the parasite (1889–1891); and there Theobald Smith, working on Texas fever of cattle, discovered the parasite to be a protozoan inhabiting the red blood corpuscles and he and Kilbourn demonstrated that the carrier of the disease from infected to healthy cattle was a blood-sucking tick (1889–1893). Following these leads, the writer in 1895 succeeded in transmitting the bacterial wilt of cucurbits by means of the cucumber beetle (*Diabrotica vittata*), and in 1896 the bacterial brown rot of the potato by means of the Colorado potato beetle. The vast development of this subject belong to a later decade.

Let us turn now to the human and animal side of pathology.

In 1887, David Bruce, an English army officer, isolated the micrococcus of Malta fever. In 1887 Schütz discovered in a coccus the cause of contagious pleuropneumonia in horses.

In 1887, 1891, 1894, Émile Roux published on immunity against symptomatic anthrax.

In 1889 Roux and Yersin definitely proved the existence of a filterable toxine in diphtheria and paved the way for Emil von Behring's *antitoxin*, by use of which the diphtheria death-rate has been definitely lowered. Immediately after its discovery Roux demonstrated on several hundred children in the hospitals of Paris the efficacy of this antitoxin and reported upon it at the Budapest Congress.

Following Koch's work, tuberculosis was discovered in birds and in various quadrupeds, and following Lavaran's work malarial parasites were found in the blood of quadrupeds, birds, and reptiles. Coccidial diseases also were discovered in man and in various animals.

In 1890 Koch discovered tuberculin by means of which early diagnoses of tuberculosis can be made but the great hope of a cure from its use, which filled the public mind for a time, was doomed to bitter disappointment.

In 1890 tetanus antitoxin was developed by Behring and Kitasato.

In 1891 Councilman and Lafleur, at Johns Hopkins in Baltimore, following earlier studies of Lösch in Russia (1875) and Kartulis in Egypt (1885), showed clearly that one form of dysentery is due to an amoeba.

In 1893 the plague appeared in Hong Kong and in 1894 Kitasato and Yersin, independently, announced the discovery of the plague bacillus.

In 1892 Theobald Smith discovered serum anaphylaxis and in 1894 the protozoan cause of infectious enterohepatitis in turkeys and the following year published a full account of it.

In 1894 appeared the first edition of Novy's "Laboratory Work in Bacteriology."

In 1895 V. A. Moore differentiated infectious leukaemia of fowls from chicken cholera.

During this decade Pfeiffer (1892) isolated and described the influenza bacillus. In this decade (1896) it was discovered that immunity against typhoid fever could be induced by the inoculation of sterilized cultures (Pfeiffer and Kolle).

In 1887 the "Centralblatt für Bakteriologie und Parasitenkunde" was founded to become a vast storehouse of the new knowledge and in the last year of the decade (1896) appeared the fine, two-volume, third edition, of Flügge's "Microörganismen mit besonderen Berücksichtigung der Aetiologie der Infektionskrankheiten" on which several authors collaborated. This work of 30 years ago describes 161 kinds of bacteria and shows to what an extent pathologists and bacteriologists had already multiplied and become interested in culture methods and classifications. Already 22 groups of the rod-shaped bacteria were recognized, of which at least 16 groups contained parasites; not to mention Coccaceae and Spirillaceae, in which groups also parasites had been found; not to mention also Protozoans. The latter were divided into 4 main groups each containing parasites. Nothing, perhaps, in brief, gives a better idea of the progress of pathology during the first 2 decades of our 50 years than an enumeration of the then-recognized classes of the rod-shaped bacteria. Flügge's groups of these bacteria are named from prominent organisms in each group and those containing parasites are as follows:

The hay-bacillus group, the anthrax group, the oedema bacillus group, the symptomatic anthrax group, the tetanus group, the proteus group, the fluorescent bacilli, the water bacilli (here plant parasites), the *Bacillus aërogenes* group, the *Bacillus coli* and typhoid group, the hemorrhagic septicaemia group (here mouse typhus), the *Bacillus sputigenes* group (here mouse plague), the influenza bacillus group, the swine measles group, the glanders group, the diphtheria group, and the tubercle group. At the end Flügge has a catch-all for diseases of uncertain origin, variously ascribed to bacteria, but all imperfectly un derstood and requiring further investigation: such diseases as rabies, smallpox, measles, typhus fever, whooping cough, yellow fever, beri-beri, and trachoma.

1897–1906

We come now to the third decade. It was easy to weave the first part of this discourse because only a few striking threads entered into its composition. It has been difficult to make this third part; so many strands have necessarily

entered into it. Only one who has read widely in literature knows what an outburst of energy followed the work of the great masters in every field of pathological research. The subject will get more and more complex and finally become unmanageable as we go on. Only a much vaster loom would suffice to weave it. I can but hope to select a few bright threads here and there for my very modest fabric.

In 1897 Arthur recommended treatment of seed tubers with formalin for prevention of potato scab.

In 1896 to 1905, Rudolph Aderhold, in Germany, greatly advanced our knowledge of the *Fusicladium* scab of apples and pears.

In 1897 Eriksson announced his mycoplasm theory of the rusts.

In 1898 Halsted wrote on asparagus rust, and during this decade that disease spread all over the United States.

In 1898 Beyerinck confirmed Iwanowski's filtration experiments and showed that the virus of tobacco mosaic without developing any visible growth would pass in 10 days from the upper to the lower layers of an agar plate. He ascribed the disease, therefore, to a "*Contagium vivum fluidum.*"

In 1898 Hollrung commenced the publication of his useful "Jahresbericht ueber das Gebiet der Pflanzenkrankheiten."

In 1898 Carleton made his first trip to Russia, bringing back the Kubanka durum wheat, resistant to heat and cold and to rust. In 1900 he went to Russia again, bringing back the Karkoff red winter wheat and various other valuable cereals. In 1899 his bulletin "Cereal rusts of the United States" was published.

In 1898 the writer described "little peach" from Michigan orchards, a disease since discovered in other states, the cause of which is still in doubt.

In 1900 Sturgis published his "Literature of Plant Diseases." In 1900 Selby published his condensed handbook of the diseases of plants in Ohio. In 1900 appeared Liberty Hyde Bailey's magnificent "Cyclopedia of American Horticulture" in 4 volumes, useful to everybody interested in plants. That year appeared also the third edition of Hartig's "Lehrbuch."

In 1900 L. R. Jones published his fine paper on the soft rot of carrot and other vegetables.

In 1900 Robert Hartig wrote on *Rosellinia quercina*, a parasite on young oak roots.

In 1900 or earlier J. C. Arthur began to work on rust fungi and has continued to do so up to the present time with many important results.

In 1900 appeared the second volume of Migula's "System der Bakterien," in which 1327 species are considered. At the present time there are probably 5,000 species names of bacteria, and no second Migula is in sight.

In 1901 Duggar and Stewart published on *Rhizoctonia* diseases.

In 1901 Karsten, in Buitenzorg, Java, further described *Cephaleuros mycoidea*, algal parasite in leaves of tropical plants, first seen by Cunningham (1879) and called *Mycoidea parasitica*.

Bolley, Arthur's student, studied in 1901 the flax *Fusarium* in North Dakota,

and afterwards flax diseases in Europe, with money from my fund, and W. A. Orton, working at first in my laboratory and afterwards independently, overcame the *Fusarium* diseases of melons and cotton in our southern states by hybridization and selection (1889–1906).

In 1902 J. B. S. Norton discovered the Sclerotinial stage of the *Monilia* of the peach, something for which many of us had looked in vain because we looked at the wrong time, that is, the first season rather than the second.

In 1902 Spieckermann, in Germany, published his fine paper on the bacterial soft rot of vegetables.

In 1902 Zimmermann found bacteria in the leaf nodules of Rubiaceae in the East Indies.

In 1902–1904 F. M. Rolfs published on the *Rhizoctonia* disease of the potato in Colorado.

In 1905 Hermann von Schrenk, of St. Louis, wrote on intumescences due to chemicals.

In 1906 appeared the second edition of Kirchner's useful handbook: "Die Krankheiten und Beschädigungen unserer landw. Kulturpflanzen."

In this decade the writer, associated with Dean B. Swingle, continued his studies of parasitic Fusaria, isolating one from the potato (1904) which subsequently received a good deal of attention from other workers. For it we revived an old name which was practically a *nomen nudum*, *F. oxysporum* Schlechtendahl.

One paper of mine (1899) dealing specifically with the fungous infestation of agricultural soils by parasitic Fusaria attacking melons, cotton, cowpeas, cabbages, tomatoes, and sweet potatoes, has been generally overlooked because it was published in an out-of-the-way place ("Scientific American Suppl." Nov. 18, 1899). Moral: When you have things to publish, put them where persons are likely to look for them!

By the beginning of this decade the ravages of the introduced San José scale had become so wide-spread and serious in the eastern United States that a representative convention of interested parties, scientific men, nurserymen, horticulturists and fruit-growers, convened in Washington in 1897, and memorialized Congress to enact a law looking toward the restriction of the unlimited and uninspected importation of nursery stock and ornamental plants, but the counter influence of importers and jobbers was too strong, and it required other sad experiences and 15 years of time to get such a bill through our Congress. After repeated introductions of bad parasites that body finally consented to act, but not until the year 1912.

In this third decade the writer had his dispute with Prof. Alfred Fischer, of the University of Leipsic, as to the existence of bacterial diseases of plants (1899–1902), and commenced the Carnegie monograph on "Bacteria in Relation to Plant Diseases" (Vol. I, 1905). In this decade L. R. Jones developed a strong center for the study of plant diseases in the University of Vermont, and many of the experiment stations appointed plant pathologists, entomologists, and animal pathologists, or enlarged the scope of those already at work.

In this decade Thaxter demonstrated that onion smut enters the plant only in the seedling stage and can be prevented by growing the seedlings past this stage in a sound seed-bed, and then transplanting to the fungus-infested fields. In this decade Bolley used formaldehyde for treatment of flax seed to overcome *Fusarium* wilt, and Woods and Dorsett used feeble doses of hydrocyanic acid gas for insect pests in greenhouses with very marked success. Formalin and mercuric chloride were also used by various persons for potato scab.

In 1903 the writer discovered *Bacterium pruni* in leaf and fruit spots on the Japanese plum, undoubtedly introduced from Japan or China, and subsequently cross-inoculated it to peaches. This is now a bad leaf disease of the peach in parts of our South, spotting the fruits and defoliating the trees in midsummer.

In 1903 Otto Appel described the black-leg of potato due to *Bacillus phytophthorus*. Various American writers have ascribed this disease to *Bacillus atrosepticus*. I am probably the only man in this country who ever had for study cultures of Van Hall's *Bacillus atrosepticus*, and it was not identical with *B. phytophthorus* nor pathogenic in my hands, nor were Van Hall's own inoculations very conclusive. *Bacillus phytophthorus*, on the contrary, will still rot potatoes after 20 years in my laboratory.

In 1903 Kohl studied *Stilbella flavida*, cause of a serious stem, leaf, and fruit disease of coffee trees. I have recently seen it in material received from Panama.

In 1903 Stevens and Sackett, in North Carolina, described the serious Granville tobacco wilt, correctly attributing it to bacteria, but leaving the species undetermined.

In 1904 Klebahn published his important book on the rusts that have more than one host: "Die Wirtswechselden Rostpilze;" and in the same year Clinton's monograph appeared on the "North American Ustilagineae."

In 1905 Scott, of the Bureau of Plant Industry, had remarkable success in preventing bitter-rot of the apple by use of the Bordeaux mixture.

This disease which caused losses estimated at $10,000,000 in 1900, and very great losses in the years immediately following, was controlled in his experiments to the extent of 93 to 98 per cent. Expressed in bushels, the sprayed trees (those that received 5 or more treatments at the proper time) yielded 50 to 60 bushels of sound apples, while all the apples rotted on the control trees, that is, *A* bore 1 sound apple, *B*, 6 sound apples, and *D*, 2 sound apples. The fungus *Glomerella*, cause of this rot, was extensively studied by Hermann von Schrenk, later by many others.

In 1905 appeared the first and only installment of Farlow's "Bibliographical Index of North American Fungi."

In 1901 to 1906 appeared papers on parasitic Gloeosporiums by various European writers (Beauverie, Viala and Pacottet, Laubert, and Wagner).

In 1906 Ralph E. Smith, in California, described a brown rot of lemons due to a peronosporaceous fungus, called by him *Pythiacystis* nov. gen. but said by Leonian to be only another *Phytophthora*.

Studies in Europe were largely devoted to fungous diseases of plants.

General attention was turned, during this decade, to the possibility of over-

coming plant diseases through hybridization and selection, and many persons made experiments. Galloway and Dorsett, for example, showed that a serious hothouse disease of violets (the *Alternaria* leaf spot) could be overcome in this way, and Millardet in southern France made many crosses between *Vitis vinifera* and hardy American vines, to obtain resistance to *Phylloxera*, in which he was successful. In 1906 Biffen received a gold medal from the Royal Horticultural Society in London for originating a variety of wheat resistant to rust. Pierce in California successfully crossed raisin grapes to resist *coulure*, the ragged bunch disease, and attempted with partial success to get walnut hybrids resistant to his bacterial disease of walnuts. This subject, as we shall see, has increased in importance with every decade and the end is not yet. It offers good prospect for the final victory over some of our worst plant diseases but the time and labor involved in creating and testing resistant hybrids and obtaining good commercial sorts are very great.

Useful books multiplied, and the literature of plant diseases and of animal diseases began to take on its modern aspect. In fact, in this third decade and in the following decades, as I have said, so many persons in so many countries published papers on plant diseases and animal diseases that it is hopeless to try to enumerate all the good papers. I can only mention here and there one, endeavoring in general to select those of conspicuous merit or those that opened up new paths.

In 1901 Marshall Ward published his book on "Disease in Plants" and in 1903 Küster published the first edition of his "Pathologische Pflanzenatomie." The same year (1903) Sydow began the publication of "Annales Mycologici" with many collaborators.

The great danger to the United States from parasites brought in on foreign plants already began to be generally recognized, and introduction gardens were established by the United States Department of Agriculture where its own introduced plants could be watched until they were known to be free from dangerous insects and fungi, before a general distribution was made. One of these gardens is at Miami, Florida, and another at Chico, California.

By 1906 the San José scale was widely prevalent in the eastern United States, the chestnut bark disease (discovered in 1904) was attracting general attention on Long Island, and pear blight had been for some years destructive in California, having finally been introduced from the eastern United States, where it had been present in orchards for more than a century and where it was present probably before the first settlers arrived, native on wild species.

Let us turn now for a few moments to human and animal diseases.

In 1898 Nocard and Roux isolated an exceedingly minute sometimes filterable micro-organism from the contagious pleuropneumonia of cattle by growing it in collodion sacks in a mixture of serum and bouillon in the abdominal cavity of rabbits and with it reproduced the disease. The nature of this organism is still in doubt. It looks something like a small coccus.

In 1898 Shiga discovered the dysentery bacillus, common in Japan and many other countries, and various strains of this dysentery bacillus have since been

distinguished. That year Theobald Smith showed that the bovine types of tubercle bacilli are distinct.

In 1900 a fungus, identified by the writer as *Sporotricum*, was found, by Schenk of Johns Hopkins, to be the cause of chronic human abscesses, and since that time several hundred cases have been observed and a considerable literature has developed, mostly American and French. The parasite enters through wounds, often very slight ones, and the remedy is heavy doses of potassium iodide. Many other fungous parasites occur on man and other vertebrates, and they are nowhere studied at present as they should be. Thaxter has monographed those occurring on insects and arachnids (the Laboulbeniales).

In 1900 in Cuba a United States commission, consisting of Drs. Reed, Carroll, Agramonte, and Lazear, demonstrated the insect transmission of yellow fever. Sternberg, the bacteriologist, was Surgeon General at the time and without his warm support nothing would or could have been done.

In 1901–1902 Welch and Nuttall, of Johns Hopkins, described their anäerobic gas oedema bacillus (designated *Bacterium welchii* by Migula), type of a group causing deadly wound-diseases, much in evidence during the late World War.

In 1903 Novy, one of my classmates, with McNeal (University of Michigan) demonstrated that *Trepanosoma lewisi* from the blood of a rat and *T. brucei* from the blood of a dog brought from Africa, could be cultivated on blood agar, thus greatly advancing the study of protozoan blood parasites.

In 1903, De Schweinitz and Dorsett, of the Bureau of Animal Industry of the Department of Agriculture in Washington, determined hog cholera to be due to a filterable virus, and Dorsett with other colleagues published on it again in 1905.

In 1903 Negri discovered the "Negri bodies." They are constantly present in hydrophobia in the ganglion cells, have not been seen in any other disease and are now generally believed to be consistently associated with rabies, but have not been cultivated, so far as I know.

In 1902–1904 appeared (in 4 volumes) the great German work of Kolle and Wassermann on infectious diseases of man and animals.

In 1905, Schaudinn and Hoffman in Germany, discovered the cause of syphilis, a flagellate protozoan, now called *Treponema pallidum*. The following year the Wassermann reaction was announced for the detection of the disease, and this test is now in general use all over the world.

In 1905, following Schaudinn's discoveries, Castellani discovered in a flagellate protozoan the cause of that dreadful tropical disease "Yaws," and clearly distinguished it from syphilis. Stovarsol is said to be an effective remedy.

1907–1916

The fourth decade was one of great activity in the study of plant diseases in the United States.

During this decade L. R. Jones established a strong center of plant pathological research at the University of Wisconsin, and H. H. Whetzel another

at Cornell University. Bessey taught general botany including mycology in Nebraska, and Thaxter and Farlow taught mycology at Harvard. Their students are now everywhere in the United States.

In 1907 Shear published upon *Ozonium omnivorum*, cause of a root-rot of cotton and alfalfa in Texas and Oklahoma. Reddick in New York was then (1907–08) publishing upon grape diseases.

In 1907 Townsend, Nellie A. Brown, and myself, working together, discovered the cause of crowngall, which on account of its structure and manner of growth, the writer likened to malignant human and animal tumors. This discovery led to various modifications in nursery and orchard practice and to a system of rigid inspection of shipments, good for the planter but irksome to nurserymen and importers, who are now trying to break it down.

In 1907 Aderhold and Ruhland, in Germany, described a gum disease of cherry trees due to *Bacillus spongiosus*. Later F. L. Griffin found this in Oregon and Barss studied it.

In 1908 the writer determined the Granville tobacco wilt to be due to *Bact. solanacearum*, and the same year described the morphology and cultural characters of the olive tubercle organism. In 1909 he also described the Grand Rapids tomato disease, a phloem wilt disease, due to a yellow schizomycete, and in 1910 published a verification of Appel's work on black-leg of the potato with cultures of *Bacillus phytophthorus* received from Rudolph Aderhold in 1906.

In 1910 the writer also called attention to another parasitic soil *Fusarium* isolated from the blackened vascular bundles of a badly diseased Cuban banana. This disease, due to *Fusarium cubense*, and now commonly known as the "Panama disease," is very destructive, has belted the globe and bids fair to give banana growers a great deal of trouble for a long time to come. Some varieties are immune but these are not good commercial varieties, and the sterility of the best bananas makes it difficult to obtain hybrids. Brandes, Carleton, and Reinking have all confirmed the pathogenic nature of this *Fusarium*. Ashby has reported it from Trinidad and Ocfemia has found it in the Philippines. In transplanting bananas the utmost care should be taken to avoid carrying the organism from diseased to healthy fields on root stocks, on tools, or on the feet of men and domestic animals; drainage from old to new plantations should also be avoided. In planting new fields it would be best to use an entirely new set of teams, tools, and laborers.

In 1908 the American Phytopathological Society was founded, holding its first meeting in Boston in 1909, and in 1911 it began the publication of "Phytopathology." Prof. L. R. Jones had much to do with the founding of this society and with the beginning of its journal.

Literature increased enormously during this decade.

In 1908–9 Delacroix and Maublanc published their "Maladies parasitaires des Plantes cultivées."

In 1909 appeared B. M. Duggar's textbook on "Fungous Diseases of Plants," the first comprehensive American textbook. One has only to compare this with Scribner's little book to see what great advances were made in ten years.

In 1910 Selby published his Bulletin 214: "A brief Handbook of the Diseases of Cultivated Plants in Ohio."

In 1910 appeared Appel and Wollenweber's monograph of the genus *Fusarium*.

In 1910 appeared the first edition of Massee's "Diseases of Cultivated Plants and Trees."

In 1909 to 1913 appeared the first edition of Ferraris' Italian handbook of plant diseases: "I Parasiti vegetale delle piante coltivate od utile."

In 1911 appeared Delacroix and Maublanc's "Maladies des Plantes cultivées dans les Pays chauds," the first handbook of tropical plant diseases.

In 1911 appeared the second volume, and in 1914 the third volume, of the writer's Carnegie monograph: "Bacteria in Relation to Plant Diseases."

In 1911 Miehe, the German, found bacteria in the leaf teeth of *Ardisia*.

In 1912 Klebahn published his "Grundzüge der allgemeine Phytopathologie."

In 1912–1913 Carl Hartley recommended sulphuric acid for damping-off of conifers in seed-beds, but it was used earlier on sugar-beet seed by Hiltner ("O. U. Zeits. f. Zuckerind. u. Landw." 1899), and has been used since to free cotton seeds from *Bact. malvacearum* (Ludwig, 1922).

In 1913 Leonard Lee Harter described foot-rot, a new disease of the sweet potato, and in 1913, 1914, and 1915, he published on stem rot of the sweet potato.

In 1913 and earlier, J. B. Norton of the Bureau of Plant Industry bred asparagus plants resistant to the destructive rust fungus.

In 1913 Gloyer used formaldehyde on seed potatoes to prevent *Rhizoctonia*.

In 1913 appeared Stevens' "Fungi Which Cause Plant Diseases," and Melville T. Cook's "The Diseases of Tropical Plants."

In 1914 Spieckermann and Kotthoff, in Germany, described the bacterial ring disease of the potato. That year the French Société de Pathologie Végétale was organized.

In 1914 Morse and Shapovalov wrote on the potato *Rhizoctonia* in Maine.

In 1915 Clara Hasse, of the Department of Agriculture in Washington, announced the discovery of the cause of citrus canker (*Bact. citri*). The same year F. M. Rolfs published on the black spot of stone fruits due to *Bact. pruni*.

In 1916 Peltier (Illinois Bull. 189) wrote on parasitic Rhizoctonias in America. That year Burrill died.

In 1916 V. B. Stewart wrote on the *Phyllosticta* disease of the horse-chestnut.

In this decade (1910) Němec, of Prague, made interesting studies of the plant tumors due to the nematode *Heterodera radicicola*, finding the enormously enlarged tumor cells near the head of the worm to contain 50 to 150 nuclei.

In this and the following decade bean diseases attracted a good deal of attention in the United States, and Barrus obtained a red kidney bean resistant to anthracnose (1915).

In 1915 L. R. Jones and Gilman demonstrated that the *Fusarium* disease of cabbage then seriously prevalent in Wisconsin, could be controlled by the breeding and selection of resistant plants, a brilliant piece of work.

In 1916 appeared the second edition of Küster's "Pathologische Pflanzen Anatomie" (3rd ed. 1925).

On the human and animal side the following are some of the landmarks of the fourth decade:

In 1907 Halberstädter and S. von Prowazek, and independently Greeff, discovered the very minute trachoma bodies in epithelial cells of the conjunctiva in cases of trachoma, not yet cultivated.

In 1908 V. A. Moore, of Cornell University, published the third revised edition of his "Pathology and Differential Diagnosis of the Infectious Diseases of Animals."

In 1908 appeared the third edition of Hewlett's "Manual of Bacteriology."

In 1909 Harold Taylor Ricketts discovered the cause of the Rocky Mountain spotted fever, isolating the organism since known as *Rickettsia* from man and from the ticks which he had previously proved to be carriers of the disease, also from the eggs of the ticks.

In 1909, by intracranial inoculation of tissue from diseased spinal cords, Flexner and Lewis produced infantile paralysis in monkeys and transmitted it from one monkey to another through a long series. Eighty-one monkeys were infected with the virus and the average incubation period was 9.82 days.

In 1909 appeared Franz Doflein's splendid "Lehrbuch der Protozoenkunde," this being the second edition of his "Protozoen als Parasiten und Krankheitserreger." Other editions were issued in 1911 and 1916. Doflein was also a romance writer, musician, artist, and poet.

In 1909 after a long series of experiments, Paul Ehrlich discovered salvarsan, called also 606, an organic arsenic compound, a partial remedy for syphilis.

In 1907–1909 appeared 2 supplementary volumes of Kolle and Wassermann's "Handbuch der pathogenen Mikroorganismen," and in 1912–1913, a new 8-volume edition by Kolle and Abel.

In 1910 appeared the 5th edition of Muir and Ritchie's "Manual of Bacteriology."

In 1910 appeared the first edition of Hiss and Zinnser's "Textbook of Bacteriology."

In 1910 Borrel, in France, discovered the rat-liver-cyst sarcoma, associated with the larval form of a tapeworm.

In 1910, in the Philippines, by inoculating diseased material containing the *Treponema pertenue*, Henry J. Nichols produced Yaws in a monkey, and from this monkey transmitted the disease to three generations of rabbits.

In 1910 Peyton Rous published his first paper on: "A transmissible avian neoplasm" (sarcoma of the common fowl).

In 1911 Hideo Noguchi, of the Rockefeller Institute in New York, first isolated in pure culture *Treponema pallidum*, the cause of syphilis.

In 1911 appeared the first edition of Marshall's "Microbiology," on which many men collaborated.

In 1911 McCoy, in California, now Director of the Hygienic Laboratory in

Washington, discovered *Bacterium tularense* in ground squirrels dead of an epidemic disease resembling plague, which he was then studying.

In 1912 appeared S. von Prowazek's "Handbuch der Pathogenen Protozoen."

In 1913 Flexner and Noguchi cultivated from the brains of children dead of infantile paralysis a very minute, filter-passing, coccus-like organism, about 0.2 μ in diameter, with which they reproduced the disease in monkeys from the brains of which they reisolated the organism.

In 1913 Fibiger of Copenhagen produced carcinoma in the stomach of rats by feeding them nematodes taken from the muscles of a West Indian cockroach.

In 1913 Yamagiwa and Itchikawa, in Japan, produced cancer in the ears of rabbits by repeated tar-paintings.

Immunology began to receive more general attention in this decade, and Coca in New York commenced to publish his "Journal of Immunology" in 1916, and Manson and others were calling renewed attention to tropical diseases.

1917–1926

The last decade is fresh in the minds of everyone. It has been another period of taking stock and of refinements in every direction, but there have also been great advances. For example:

It has been discovered that inside of our parasitic species there are an uncertain number of *strains* with varying potentialities. We have not one, two, or three rusts of grain, but many; not one form of each pathogenic *Fusarium*, but many forms; not one typhoid germ, but various paratyphoid organisms; not one dysentery bacillus, but several; not one crowngall strain, but several; and so on.

And what changed concepts of the bacteria have come into our minds in recent years! At one end of our gamut is Koch's old idea of the fixity of species and at the other end Löhnis' nightmare. The truth lies somewhere between the two, but it is not to be discovered in old literature, nor by peering down the barrels of microscopes at old messes below (contaminations are too easy), but rather by exact methods of isolation and cultivation. Only then can we know what all these figured globes, granules, spore-sacks, threads, and plasms really are. They may all form parts of one complex organism, and then again they may not. It is a fine piece of work for the *best men* of the next generation.[3]

In recent years there has been a great revival of interest in "virus" diseases of plants. Known first from tobacco, they have been discovered in the potato and in a great variety of cultivated plants where they had hitherto been overlooked or had attracted but little attention, probably because they were not then so prevalent. They are now recognized as among our worst plant diseases and the obscurity of their origin renders them doubly interesting. In the United States there have been five principal centers of research; Allard, Schulz, and others in Washington, Doolittle, Johnson, and others at Madison, Carsner in

[3] I have found one "spore sack," that of Glover's "cancer germ," to be the conidia of *Penicillium brevicaule*, a contaminating fungus. The rest of the "germ" was made up of several types of sporulating bacteria.

the far west, Duggar in St. Louis, and Kunkel and others in the Boyce Thompson Institute, at Yonkers-on-the-Hudson. Up to this time the cause or causes remain in doubt, but much has been learned respecting signs of these diseases, host plants, and methods of transmission. Scepticism will not down until these assumed flagellates and amoebae can be cultivated on artificial media and re-inoculated with production of the disease. Allard (1914–1918) showed that tobacco mosaic is spread by aphides, and we now know that many of these diseases are introduced by the bites or punctures of insects, that some of them have several host plants and that in some of these plants they produce no signs.

Flagellate protozoan diseases also have been discovered in plants and there is now quite a literature. Possibly, in the end, we shall find that these two groups are one. So far, trypanosome-like flagellates have been found principally in plants with a milky juice. Attention was first called to these parasites by Lafont in Mauritius in 1909. He discovered them in Euphorbiaceae and pointed out that they were transmitted by insects, but most of the work has been done in the last six years in Portugal, Italy, Russia, Dahomey, and the United States. All of the workers are agreed that the *Leptomonas* is transmitted by punctures of bugs, and several observers have seen the protozoans inside of the bugs. They are first in the intestine and later large aggregations are found in the salivary glands, from which they escape into the plants. They live over winter in certain insects (*Stenocephalus*). Euphorbiaceae, Asclepiads, Apocynaceae, Sapotaceae, Artocarpeae, Urticaceae, Menispermaceae, and Anacardiaceae have all been found to contain protozoan parasites. Franchini states that he has also found motile amoebae in the milk vessels of lettuce collected near Bologna. All of which facts are exceedingly interesting when we consider that some of the worst human and animal diseases are due to flagellate protozoans. The last paper I have seen is by Sophie H. Eckerson ("Bot. Gazette," April, 1926) in which she describes and figures small flagellate protozoans as the cause of tomato mosaic where she says they attack, honeycomb, and destroy the chloroplasts. She has also figured them from mosaic diseases of Hippeastrum, wheat, dahlia, and squash.[4]

On the plant side the following are some additional happenings of this period:

In 1917 Jagger transmitted cucumber mosaic diseases by rubbing crushed leaves of diseased plants on healthy plants.

In 1917 C. A. Scott, of Kansas, developed a practical method of control of damping-off of coniferous seedlings by treating the beds with high-pressure steam. This method, however, was in use earlier among Boston lettuce growers.

In 1917–1918 the writer and his assistants worked on the bacterial black chaff

[4] Since this was written Kunkel has published an important paper on Aster Yellows, a disease transmitted by the leaf-hopper *Cicadula sexnotata* to many kinds of plants when fed on diseased China asters, but only after an incubation period in the insect of some days ("Am. Jour. Botany," Dec. 1926). See also "Bull. Torrey Bot. Club," Nov. 1926, for a careful paper by Bessie Goldstein. In the British "Jour. of Exper. Pathology," Oct. 1926, are two papers on ultra-microscopic viruses of animals with numerous figures and literature citations. See also Contiere "Choses *Infra-Visible*" in "*Biologie Medicale.*"

of wheat. The parasite is carried over on the seed from one crop to the next and seed grain should not be obtained from diseased fields.

In 1918 McClintock and L. B. Smith published on a destructive mosaic blight of spinach and showed that it is not only transmitted by aphids, but also from one generation of aphides to another. L. B. Smith (1920) bred spinach resistant to this disease.

In 1918 Burkholder obtained a white marrow bean resistant to anthracnose.

In 1918 Halsted died and in 1919 Farlow died.

In 1919, 1920, 1923, Doolittle added much to our knowledge of cucurbit mosaic, which is transmitted by various insects. Wild cucurbits harbor the virus and one of the principal agents in its transfer is the striped cucumber beetle (*Diabrotica vittata*). The last paper is by M. N. Walker ("Phytopathology," July, 1926).

In this decade onion smut was controlled in field experiments by the use of formaldehyde (Walker, 1920; Osmun and Anderson, 1923).

In 1920 L. R. Jones, Walker, and Tisdale, continuing the work of Jones and Gilman, obtained by further hybridization and selection excellent commercial varieties of cabbage perfectly developed in fields where all the common sorts died from the attacks of *Fusarium conglutinans*. Their beautiful photographs, showing long rows of well-headed and healthy cabbage plants surrounded by thousands of dead and dying ones are very striking.

In 1923 Miss G. Wilbrink in Java demonstrated that sugar-cane cuttings could be freed from Sereh by exposure to warm water (30 min. at 45°C, followed by 30 min. at 50° to 53°C). From Sereh-diseased canes treated in this manner healthy plants can be grown. A great triumph! The cause of the disease is still in doubt.

In 1923 Agnes Quirk and Edna Fawcett, of my laboratory, published their studies on the hydrogen-ion concentration of culture media as compared with Fuller scale readings ("Jour. Infect. Diseases," Vol. 33, No. 1). Here are given the approximate ranges of growth (acid-alkaline) of 28 bacteria pathogenic to plants.

In 1925 Janczewski reported the black chaff of wheat from many places in Russia, (Vide "Science," Mar. 19, 1926, p. 305.)

In 1925 Lucia McCulloch, of my laboratory, described the cultural characters of *Aplanobacter insidiosum*, the cause of a serious vascular disease of alfalfa, now an important crop in the United States. This disease was discovered and its etiology established by Fred Reuel Jones in Wisconsin in 1923–1924.

In 1922–1926 Florence Hedges, of my laboratory, discovered and described a new and destructive bean disease due to *Bact. flaccumfaciens*. This is widespread in the United States and she also found it in Europe last year. It is carried over from year to year on and in the seeds.

INTRODUCED PARASITES

During this decade the white pine blister rust introduced from north Europe has done great damage to our pine forests.

The fungus of the chestnut bark disease introduced from Japan and beginning in a small way on Long Island twenty-odd years ago, has swept over the whole northeastern United States and destroyed our chestnut forests. Today the chestnut (*Castanea vesca*) is a thing of the past except on the outfringes of its distribution and even there it is being attacked. In all probability another 10 years will see the end of this magnificent species with the exception of an isolated specimen here and there. Our chestnut has shown surprisingly little resistance to this new enemy. Our U. S. Dept. of Agriculture is now experimenting with a very resistant Chinese variety. The fungus occurs on it but does not make any headway.

The bacterial black chaff of wheat introduced from Russia is widespread in wheat fields beyond the Mississippi, reducing yields and deteriorating good grain.

The potato wart disease, introduced from northern Europe and very destructive there in places, has gained a foothold in Pennsylvania. In Germany varieties of potatoes resistant to this disease are now being produced.

The bacterial citrus canker introduced from Japan or China by way of Mexico has damaged the citrus industry of Florida to the extent of many thousands of dollars. Indeed, at one time it bid fair to render all the citrus groves of Florida unprofitable, and would have done so but for the most strenuous efforts on the part of the state and the nation. In Florida a corps of inspectors is still required (28 men for the quarter ending June 30, 1926). During this quarter they inspected, in round numbers, 3 million citrus trees in groves and nearly 29 million citrus trees in nurseries without finding any of the canker. Since 1914 when the inspection began, ctirus canker has been found in 25 counties in Florida, on 512 estates, in 15,156 grove trees and in 342,260 nursery trees, all of which have been destroyed, mostly by means of the gasoline torch.

The boll weevil of cotton introduced into Texas from Mexico has gradually spread to the Atlantic seaboard. This vexatious pest, very hard to control, has greatly reduced the yield of cotton in our southern states, and more than doubled the cost of cotton goods.

The Japanese iris beetle, introduced on a miserable little importation of iris, has become widespread in central New Jersey and eastern Pennsylvania. In spite of restrictive measures it is spreading over into other states, appears to be free from enemies, and perfectly at home, multiplies by the million, and consumes the leaves and fruits of all orchard trees and many other plants voraciously, so as to be a great menace to our orchards, gardens, and lawns.

These are examples only. There are others I might mention.

Strenuous efforts have been made and are being made with much expenditure of state and national funds to control these pests, and, as we have seen, the citrus canker in Florida is now very well under control, at a cost of half a million dollars, but the chestnut is doomed, and it is problematic whether some of the other pests I have mentioned can be controlled. It is much easier to keep out a parasite than to control it when once introduced.

During this period the hop mildew suddenly appeared in England, France, and Germany and has done much damage. One year (an unusual spring) the

tobacco mildew appeared in Florida both in the seed-beds and in the field; but it has not been reported since. In neither of these instances do I think we must necessarily predicate recent importation, since the parasites may have been already in Europe and in the United States, on wild plants gradually multiplying and awaiting favorable conditions for attacking hop and tobacco. French writers think the hop parasite was imported on hops from Japan and spread by the wind. The greatest care should be exercised by all pathologists in the handling of plants attacked by parasites, and all such material should be incinerated when it has served its purpose, not thrown on a dump heap.

During this period several bacterial leaf spots of tobacco have been described from the United States and one of them, known as "wild fire," first described by Wolf and Foster (1917–1918) is capable of doing, and has since done, great damage to tobacco plantations. If the weather conditions are favorable, it runs over a field quickly and ruins wrapper leaves. The rapidity with which this disease has spread in all the principal tobacco districts from Massachusetts to Florida is surprising. Where was it in the earlier decades of this period? Is it an introduced species? Or, has it been here for a long time and our tobacco become susceptible to it only in recent years?—These are questions for which we have no immediate answers.

During this decade a great attempt has been made in the United States to control stem rust of wheat (our worst disease) in a dozen or more of our western states by a united effort to eradicate all the barberry bushes over a territory a thousand miles square. It is too early to know what the final results will be, but thus far they appear to be favorable. Plant breeding would seem to offer a more effective remedy. This also is being tried. Possibly dusting by airplane with colloidal sulphur is the coming remedy.

In this decade F. C. Reimer, in Oregon, made extensive efforts to find good pear stocks resistant to fire blight and in this he has been measurably successful, using certain Asiatic species of *Pyrus*, collected by Frank Meyer and himself in Manchuria.

In this decade the literature of plant and animal diseases and their parasites continued to multiply.

In 1918 appeared Whetzel's "History of Phytopathology."

In 1920 appeared the first textbook on "Bacterial Diseases of Plants," by the writer, and in 1926 we are promised a second one by Jones of Canada.[5]

In 1921 appeared the second edition of "Diseases of Economic Plants" by Stevens and Hall, revised by Stevens.

In 1923 appeared the third edition of Kirchner's handbook: "Krankheiten u. Beschädigungen unserer landw. Kulturpflanzen."

In 1925 appeared Stevens' "Plant Disease Fungi" and Whetzel's "Outlines of plant Pathology" (2d edition).

In 1920 Saccardo died, and in 1925 appeared the 23d volume of his "Sylloge Fungorum," edited by his son-in-law, Trotter. As showing the vast develop-

[5] This is now promised for 1927.

ment of mycology in 50 years, I may mention that the entire 23 volumes of this great work contain a total of more than 24 thousand octavo pages of condensed (Latin) descriptions of fungi many of which are parasites.

In 1925 appeared the thin-paper 3-volume edition of L. H. Bailey's "Standard Cyclopedia of Horticulture" (issued earlier in 6 volumes, 1914–1917), a magnificent piece of book-making containing 4,000 text figures and many full page plates. On this monumental work more than 400 persons collaborated. I shall have more to say about collaborations.

In 1926 appeared the first volume of the 3d edition of Ferraris' Italian "Treatise on Pathology and Therapy of Plants," and in the United States Heald's "Manual of Plant Diseases."

In this decade books on diseases of special crops other than the grape began to appear, for example, Taubenhaus' "Diseases of Truck Crops" (1918), "Diseases of Greenhouse Crops" (1920) and "Culture and Diseases of the Sweet Potato" (1923); Lex Hesler and Whetzel's "Manual of Fruit Diseases" (1917); Rankin's "Manual of Tree Diseases" (1918); Chupp's "Manual of Vegetable Garden Diseases" (1925); Fawcett and Atherton Lee's "Citrus Diseases and their Control" (1926).

On the human and animal side, the diseases of the Great War are fresh in the memory of all of us, particularly the ravages of influenza which created many a "plague-hushed thoroughfare." One day in Washington, I remember, there were 90 deaths from influenza. That was the peak of our epidemic. We abandoned the street-cars and some of us walked in the middle of the streets. It would seem as if the organism causing this disease must consist of several strains of varying virulence, or that the disease in its malignant form must be due to the symbiotic action of two microbes, one organism enabling the other to attack more virulently, or else, finally, that the so-called influenza bacillus of Pfeiffer is not at all its cause. Certainly, the last word has not been said on this mysterious disease.

We know as yet very little about bacterial symbiosis and antagonism in relation to disease, but beyond doubt there is a great deal to learn. Pathologists and surgeons learned much about wound-diseases during the world war, and they profited from that great insanity, if no one else did. The studies of anaërobic organisms of the type of *Bacillus welchii* and their germicidal treatment in wounds have been particularly interesting, and also the very practical results obtained from inoculations for the prevention of typhoid fever.

In 1919, 1920, and later, Bullock and Curtis, in New York, produced the rat-liver cyst sarcoma repeatedly (more than 1,000 times) by feeding to white rats the larvae of a tape worm of the cat.

In 1919, 1920, Francis, in Utah, isolated *Bact. tularense* from 7 human cases and 17 jackrabbits, and named the disease "Tularemia."

In 1921 Francis and Mayne proved the deer-fly (*Chrysops discalis*) to be a transmitter of the infection of Tularemia to laboratory animals. Since then this fever has been found in man in the District of Columbia and in 31 states as follows: California, Oregon, Utah, Nevada, Idaho, Wyoming, Colorado, New

Mexico, Arizona, Kansas, Iowa, Minnesota, Nebraska, Missouri, Arkansas, Ohio, Indiana, Illinois, Pennsylvania, Virginia, West Virginia, Kentucky, Tennessee, Maryland, North Carolina, South Carolina, Mississippi, Georgia, Texas, Alabama, and Florida, derived generally from handling dead (wild) rabbits. The disease is transmitted from rabbit to rabbit by the rabbit-tick, and the rabbit-louse, and from rabbits to man by the bite of the deer-fly, and of the wood-tick (*Dermacentor andersoni*). It is very readily contracted by handling dead wild rabbits or cultures of the organism. According to Francis, 18 laboratory workers, himself among the number, have contracted this disease. It occurs also in Japan in wild rabbits and in men and women from handling the same, especially skinned rabbits.

In Europe in 1917, 1918, and in this country in 1919 appeared acute epidemic encephalitis (the European and American sleeping sickness), due to a filterable virus.

In 1919 Noguchi isolated from cases of yellow fever a flagellate protozoan, *Leptospira icteroides*, the probable cause of the disease.

In 1922 appeared the 6th revised two-volume edition of "Die Experimentelle Bakteriologie und die Infektionskrankheiten mit besonderer Berücksichtigung der Immunitätslehre" by Kolle and Hetsch; and now (1926) a new (3rd edition) of Kolle and Wassermann in 10 volumes is being issued in parts.

In 1923–1924 Dick and Dick, in Chicago, discovered a *Streptococcus* in scarlet fever, constantly present and probably the cause, because behaving as the cause of the disease should behave. Earlier important work on the etiology of this disease was done by Ruth Tunnicliff.

In 1924 A. Besredka, of the Pasteur Institute in Paris, showed that bacteria deadly in certain tissues are harmless when placed in other tissues, the anthrax organism, for example. This kills when placed in the skin but is harmless when placed in wounded muscles, liver, lungs or other internal organs. This tissue resistance he has called "local immunity." It opens up a vast field for study.

One of the great advances of human medicine in this decade has been the preparation of insulin by Banting and Best, of Toronto, from animal pancreas (1922) for the treatment of diabetes. Hundreds of diabetics have been able to prolong their lives comfortably by taking daily hypodermic injections of this substance, obtained first in quantity from the pancreas of stock yard animals and now in greater amounts from the pancreas of fishes (Teleosts), and this work, be it remembered, could not have been carried through without animal experimentation.

In this decade E. C. Rosenow continued his interesting studies of facial infection, endocarditis and arthritis.

We may now turn for a few moments to consider the living carriers of disease. Beginning with the experiments of Merton B. Waite and Theobald Smith, of the United States Department of Agriculture, the one working, as I have said, on pear blight and the other on Texas fever, the subject has grown to vast proportions and has become one of the most important considerations of preventive medicine. I shall treat it without regard for names or dates (which overlap).

We now know definitely that there are human carriers of disease, that is, persons who appear to be well and yet are able to transmit deadly disease to others. Everyone has heard of Typhoid Mary, the cook who left many cases of typhoid fever in her trail. Cholera, amoebic dysentery, bacillary dysentery, diphtheria, and infantile paralysis are also distributed by persons who appear to be healthy. Fortunately, in most of these diseases only about one in 50, or one in 100, convalescents are of this type.[6] Of animal carriers there are many! The trypanosome of rats (*T. lewisi*) is transmitted by a louse; the deadly African Nagana disease of horses and cattle is transmitted by a fly; the surra disease of domestic animals in the East Indies and the Philippines is transmitted by a fly; the fatal African sleeping sickness, by the bite of another fly; trench fever, by a louse; the typhus fever of jails, ships, and camps, by a louse; the bubonic plague, from rats, marmots, and ground squirrels to man, often by fleas; stomach cancer of rats, by nematodes carried in the muscles of a cockroach; trichinosis in man, by another nematode living in the muscles of rats and hogs; the sarcoma of rat livers, by the larvae of a tapeworm of the cat; Texas fever of cattle, by a tick; the Rocky Mountain spotted fever of man, by another tick; malarial fever of man and birds, by mosquitoes; Dengue fever, by a mosquito; yellow fever, by the same mosquito (*Aëdes egypti*); the embryos of the worm *Filaria*, the cause of the enormous human overgrowth known as Elephantiasis, are also taken up and distributed by several kinds of mosquitoes; rat-bite fever is caused by a rat microbe; the Tularemia fever of man is caused by a microbe living in rabbits and it is transmitted by the bites of a deer fly and of a tick, and directly by handling dead wild rabbits; typhoid fever and cholera are transmitted by house flies; purulent ophthalmia, by flies; the Egyptian fluke disease, Bilharzia, by water snails; a similar disease of the Japanese, by another snail and by crabs; a third fluke disease of dogs, cats and man, by eating raw fish; a tumor of frogs, by a nematode living in earthworms; anaemia with dropsy in salamanders is associated with another nematode; Malta fever in man is caused by a coccus conveyed in milk from diseased goats; the spirochaete of African relapsing fever is transmitted by a tick; the cause of undulant fever, another spirochaete, is carried in the body of a louse and is transmitted by scratching; and so on. The list is a long one.

Referring again to the Rocky Mountain spotted fever, transmitted by infected ticks, Spencer and Parker, of the Hygienic Laboratory, have discovered a protective vaccine. This is made by grinding up infected ticks and injecting this fluid (to which ½ per cent phenol has been added) 3 times, 5 days apart.

There are also many carriers of plant parasites, as many as of animal diseases, and as in animals there are some very striking adaptations, for example, the relation of the striped beetle, *Diabrotica vittata*, to the bacterial wilt disease of cucurbits, worked out in detail by F. V. Rand in my laboratory. Here all the beetles, apparently, transmit the disease in the summer, but only a small portion of those that have wintered over are able to do so, that is, those only whose intestinal juices have not been able to destroy the ingested microbes. From the

[6] According to Col. Craig, whose opinion is based on findings during the late war, about one person in 10 in the United States is a carrier of the amoebic dysentery organism.

feces of these individuals come the first spring infections. In other words, these particular beetles behave exactly like human typhoid carriers. Rand lists nearly 50 plant diseases known or suspected to be transmitted by insects.

To recapitulate, on the human and animal side only, the following are a few of the greatest landmarks along the route we have just traversed:

Second decade. Discovery of transmission of Texas fever by ticks. Theobald Smith, 1889–1893. Discovery of transmission of the deadly Nagana disease of domestic animals by tsetse fly. David Bruce, 1895.

Third decade. Discovery of the transmission of malarial fever by mosquitoes. Ross, in birds, 1898; Grassi and Bignami, in man, and by another mosquito, about the same time (1898).

Discovery of transmission of yellow fever by mosquitoes (*Aëdes aegypti*). Reed, Carroll, Agramonte, and Lazear, 1900. Lazear lost his life while studying the disease in Cuba, as the result of a single mosquito bite.

Discovery of the transmission of the African sleeping sickness by a tsetse fly. Bruce and Naboro, 1903. The trypanosome of this disease was first seen in the blood of man by Dutton in 1901, and again by Castellani in the cerebrospinal fluid of blood of man in 1903.

Fourth decade. Transmission of typhus fever by the body louse. Nicolle, Comte and Conseil (1909). Confirmed in the United States in 1910, by Goldberger and Anderson, and in Mexico by Ricketts and Wilder. Ricketts lost his life in the study of this disease, another brilliant martyr to science.

Fifth decade. Proof by Siler, Hall, and Hitchens ("Philippine Jour. of Sci." Jan.–Feb. 1926) of the transmission of Dengue fever by the mosquito *Aëdes aegypti*. Further interesting discoveries and applications to human and animal welfare drawn from all of these.

I will mention only one such application. Yellow fever, once endemic in Cuba, the Panama Canal Zone, and the west coast of South America, taking thousands of lives annually, and occasionally invading and terrifying the United States, has disappeared, because all the breeding places of the mosquito carrier have been hunted out and brought under control. According to the International Health Board of the Rockefeller Foundation, which has shown great and praiseworthy activity in this and other public health directions, there has not been a case of yellow fever in Guyaquil in six years, and yet this was one of the worst places on the west coast of South America. Without mosquito control the Panama Canal could not have been built, nor could it now be maintained without great loss of life.

In human and animal pathology splendid literature continued to develop on every hand, and the public interest in the subject is shown best of all perhaps by the numerous new editions of standard works, for example, to mention only those in this country: In 1918 appeared Warthin's English revision of the 11th edition of Ziegler's "General Pathology." In 1919 appeared the 9th edition of McFarland's "Textbook on the Pathogenic Bacteria." In 1919 appeared the 1st edition, and in 1922 the 2nd edition, of James Ewing's immense "Neoplastic Diseases: A Textbook on Tumors." In 1920 appeared the 4th edition of Wells'

"Chemical Pathology." In 1921 appeared the 10th edition of Abbotts' "Principles of Bacteriology." In 1921 appeared the 2nd edition of Kendall's "Bacteriology." In 1922, 1923, appeared the first two volumes of Victor C. Vaughan's magnificent "Epidemiology and Public Health." In 1923 appeared the 4th edition of Rosenau's 'Preventive Medicine and Hygiene." The same year appeared the 7th edition of Stitt's "Practical Bacteriology, Blood-work, and Animal Parasitology, including Bacteriological Keys, Zoological Tables and Explanatory Clinical Notes." In 1924 appeared the 8th edition of Mallory and Wright's "Pathological Technic." In 1924 appeared the 8th edition of Jordan's "Bacteriology." In 1925 appeared the 8th edition of Park and Williams' "Bacteriology." In 1925 appeared the 3rd edition of MacCallum's superb "Textbook of Pathology." In 1925 appeared the 13th edition of Delafield and Prudden's "Textbook of Pathology," revised by Francis Carter Wood.

This fifth decade everywhere has been one of multiplied and multiplying books and journals and contributions on all phases of pathology and parasitology. Biological papers are now being published, it is said, in 5,000 places, and many of them are of interest to the pathologist. How shall we keep up with this flood of literature which is bound to continue and to increase in volume? Much of it is certain to be whelmed and lost under the ever-increasing output. The only papers likely to survive will be such as are written with the utmost clearness and cogency, boiled down as much as possible, based on extensive and thorough work; and, if the subject permits, well illustrated; and I may add, furnished *in every case* with a good brief summary, and, if they are books, with a respectable index. It is a disgrace to publish any book without an index.

To help out, the General Education Board of the Rockefeller Foundation has promised to finance for the next seven years "Biological Abstracts," the first number of which is due shortly, and which is designed to cover with good abstracts, as far as possible, the biological literature of the whole world.[7]

A few general considerations and I am done. I have kept you too long, but I found it impossible to compress even an imperfect summary of the doings of 50 years into as many minutes.

During this 50 years we have seen two world-wide epidemics of influenza, 1889–1890 and 1917–1918, both beginning in Asia and moving rapidly westward until they belted the globe with hundreds of thousands of deaths and with the public terrified and helpless in their grip. Such epidemics, and there have been many in the history of the world, not only of influenza but of cholera and of plague, are examples of what a parasite can do when all of the conditions for its dissemination are favorable. Such pan-demics are more disastrous than great wars, and probably are best attacked where they originate among the ignorant swarming millions of Asia, a peril to all the rest of us. In India alone in the ten years; 1901 to 1910, there were 6,563,649 deaths from *one preventable disease*, the bubonic plague.

Throughout this whole 50-year period the most strenuous efforts have been made to discover the cause of cancer in man, but so far unsuccessfully. The need

[7] Vol. I, No. 1, appeared in December, 1926.

is very great the world over. In the United States alone, according to Hoffman, carcinoma (one type only of cancer) destroys more than 80,000 persons every year, McCarty says 100,000.

Cancers in some of the lower animals, however, have been connected with the presence of parasites (nematodes, Fibiger, 1913), tapeworm larvae (Bullock and Curtis, 1920), and in case of the chicken sarcoma, with a filterable virus (Rous, 1910), which Gye and Barnard (1925) believe to be an organism. Crown-gall, or cancer in plants, if it may be so called, is due to *Bacterium tumefaciens*. Blumenthal and his associates in Berlin have also isolated what appear to be strains of this organism from human tumors and with them have produced tumors in plants and malignant tumors in rats. Very recently, Dr. Fritz Kaufmann, of Koch's Institute for Infectious Diseases in Berlin, has isolated an organism, said by him to belong to the *B. tumefaciens* group, from a carcinoma of the mouse (Ehrlich strain) and with it has produced tumors in plants. The bulk of medical opinion at present may be said to favor the view that malignant tumors in man are due to a variety of long-continued local irritations rather than to any specific parasites. Favoring this view, which I do not share, is the fact that cancers may be produced in experimental animals by tar-painting, by extracts of soot, and by other chemical substances applied repeatedly. One fact brought out clearly during the last decade is that the tendency to cancer in mice and rats, and probably also in man, is an inheritable factor, but there are other factors. Four good books containing the older views are: Roussy et Leroux, "Diagnostic des Tumeurs" (Paris, 1921); Masson, "Tumeurs—Diagnostics histologiques" (Paris, 1923); Borst, "Allgemeine Pathologie der malignen Geschwülste" (Leipzig, 1924); and Menetrier, "Cancer" (2nd ed. Paris, 1925). James Ewing's great work I have already referred to.

During this 50 years, especially in the second half of it, women have demonstrated marked ability in pathological research, not only in my laboratory and other laboratories in the Department of Agriculture, but elsewhere. The first research woman in the Department of Agriculture was Effie A. Southworth, appointed in 1887 and, I am proud to say, at my suggestion. Since then there have been many, especially in the Bureau of Plant Industry. As conspicuous examples of women pathologists outside of Washington I may mention Ruth Tunnicliff and Maude Slye in Chicago, Louise Pearce and Mayne Curtis in New York; Helen Chambers in London, Ethel M. Doidge in South Africa, Lydia Rabinowitch, Rhoda Erdmann, and Paula Meyer in Berlin, Johanna Westerdijk in Holland, and George Wilbrink in the Dutch East Indies.

This period has also seen, for the first time in the history of the world, great cooperative undertakings, public and private, for the advancement of pathology, and the suppression of disease, to some of which I have already alluded. Witness whole armies vaccinated successfully for the prevention of typhoid fever; vast territories scoured for destruction of barberries, carriers of the wheat rust, and of wild currants and gooseberries, carriers of the white pine blister rust; expeditions into Africa and Asia for the study of cholera and sleeping sickness; into South America for the study and suppression of yellow fever; and into our own

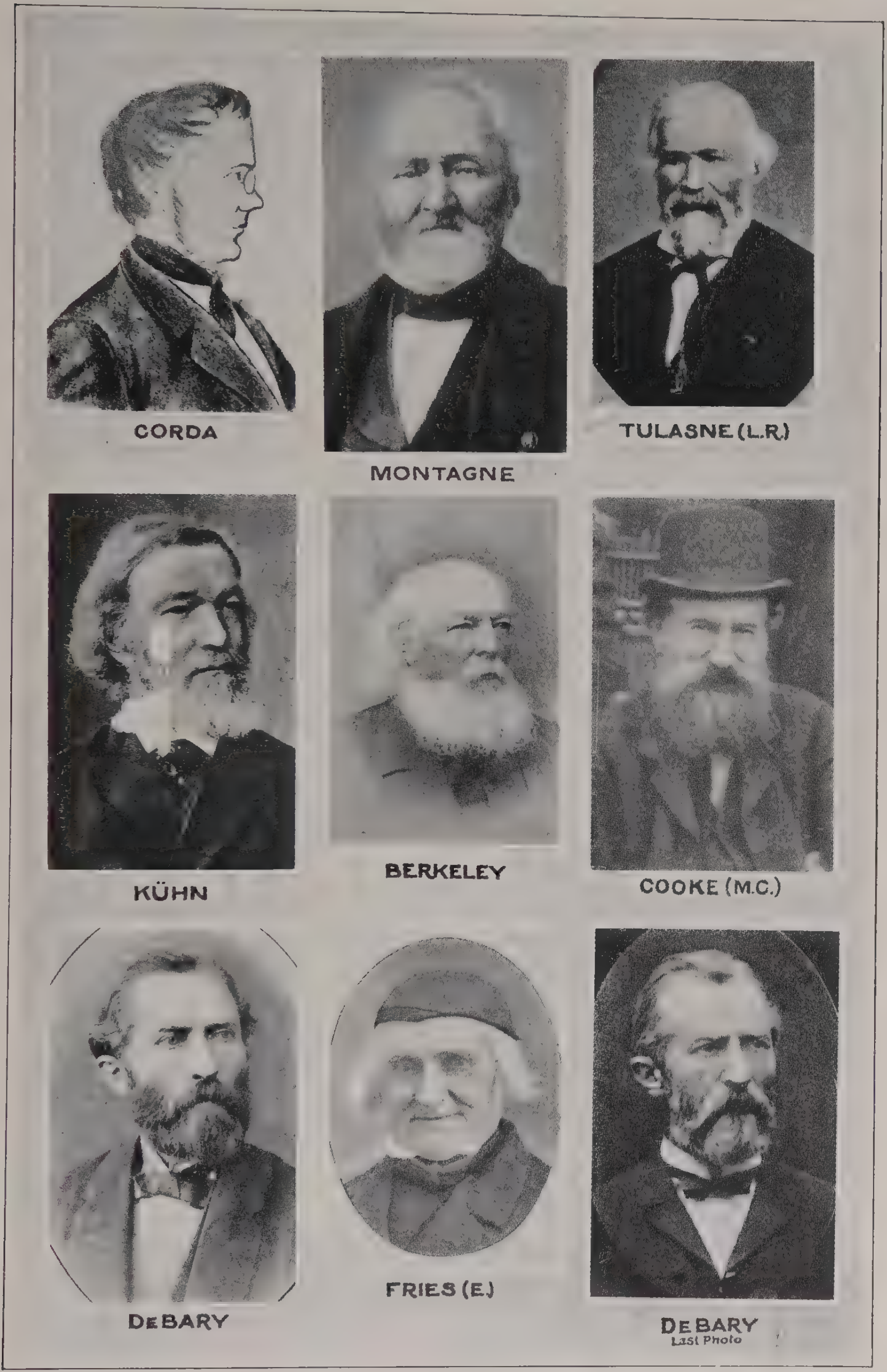
southern states and the West Indies for suppression of hook worm. Witness the founding of the Pasteur Institute and the Madame Curie Laboratories in Paris, the Lister Institute and The Institute for Medical Research in London, Koch's Institute for the Study of Infectious Diseases in Berlin, and The States Institute for Experimental Therapy in Frankfort, the Rockefeller Institute for Medical Research in New York, The Memorial Institute for Infectious Diseases in Chicago, The Oswaldo Cruz Institute in Brazil, The Boyce Thompson Institute for Plant Research at Yonkers on the Hudson, The Hygienic Laboratory in Washington, and there are also the various research laboratories in the Department of Agriculture and in the state institutions, in the Army Medical School, and in the Navy Medical School. Witness also the cooperative researches now carried on, in a smaller way, but actively, in many medical schools, in many public and private institutions for cancer research, and in many experiment stations. The outcome of all this cooperative research, as it is continued, must prove of incalculable benefit to mankind, both directly and indirectly.

Twenty-four years ago, as retiring president of the Society for Plant Morphology and Physiology, I gave an address on "Plant Pathology: A Retrospect and Prospect." I prophesied then. How well or ill, you may determine for yourselves ("Science," April 18, 1902). I will not undertake to prophesy today, but will leave this address where it stands.

We old fellows have had our day, and it is now for the younger generation, full of hope and energy, to take the field and turn and overturn. There remain many important problems to be solved, and there is much to be learned about even the best-known diseases. May the oncoming years be fruitful ones and each one of you have some share in the joy of the harvest!

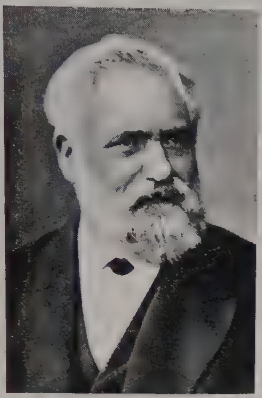
Thomas J. Burrill — George F. Atkinson
Mrs. W. G. Farlow — William G. Farlow

Smith: Fifty Years of Pathology



SMITH: FIFTY YEARS OF PATHOLOGY

Smith: Fifty Years of Pathology



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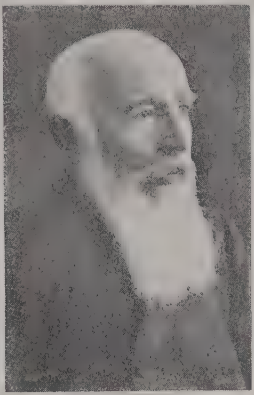

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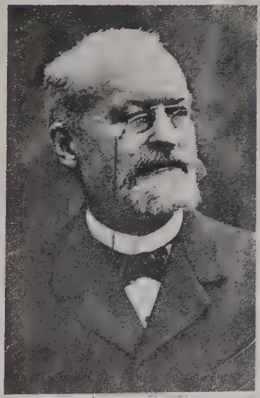

LAVARAN

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SMITH: FIFTY YEARS OF PATHOLOGY

DUCLAUX — ARLOING — CELLI

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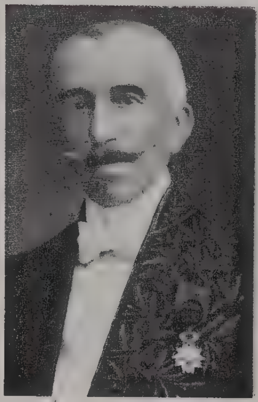
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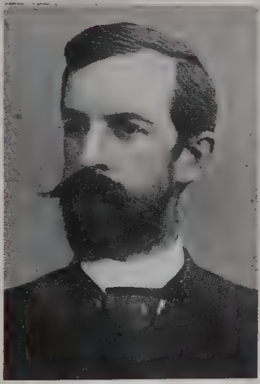
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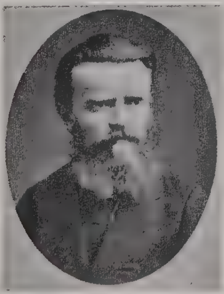
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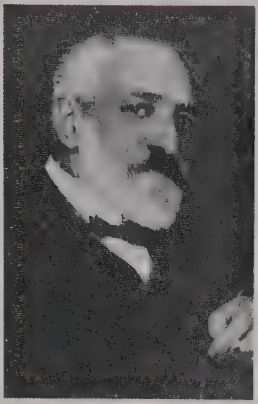
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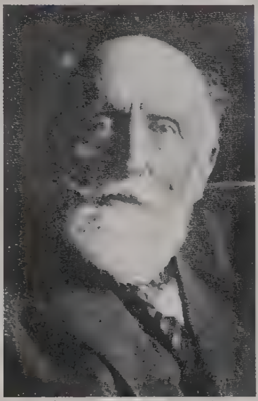
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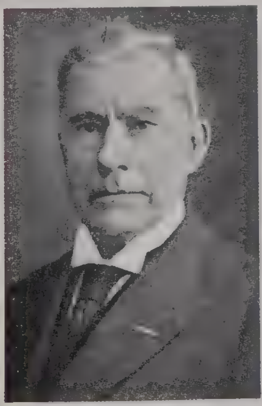
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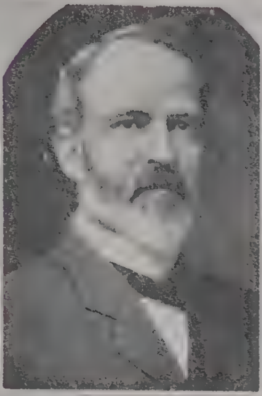

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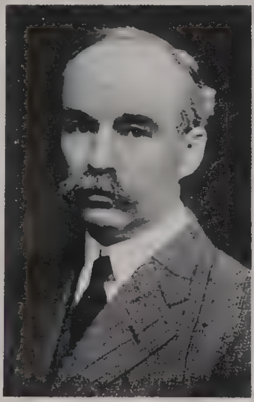

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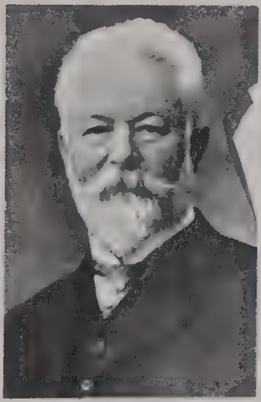

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Smith: Fifty Years of Pathology

THAXTER

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BOLLEY

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SWINGLE (D.B.)

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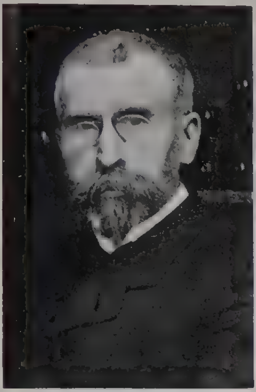
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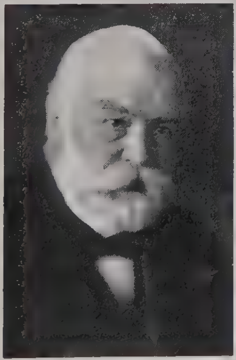
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KARSTEN (G.)

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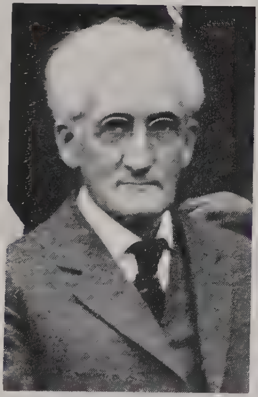

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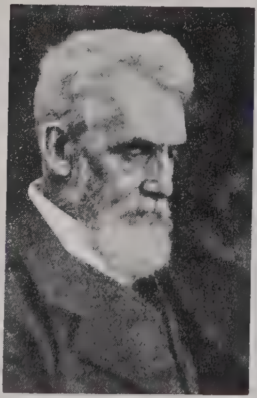

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NĚMEC

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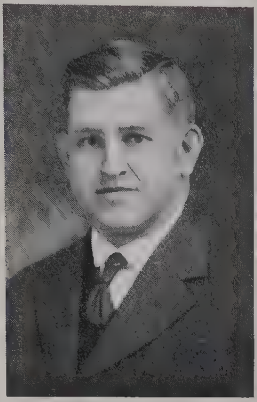

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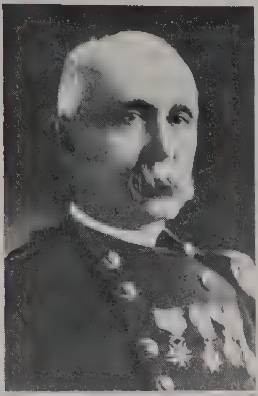
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AGRAMONTE

LAZEAR

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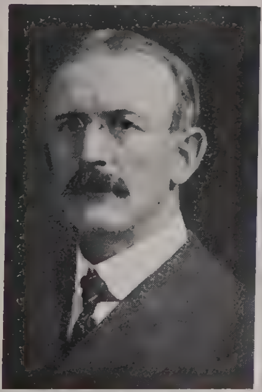

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ROLFS (F.M.)

SMITH: FIFTY YEARS OF PATHOLOGY

MASSEE

PEGLION

FERRARIS

DELACROIX

BARSS

ROCKEFELLER, Jr.

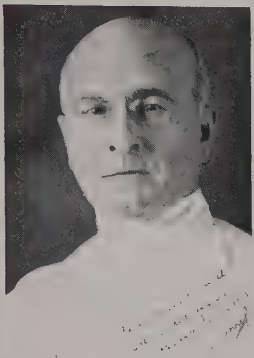
SACCARDO

PETRI

ISTVANFFI

SMITH: FIFTY YEARS OF PATHOLOGY

CRAIG



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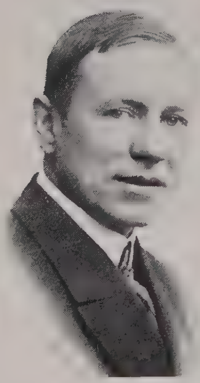

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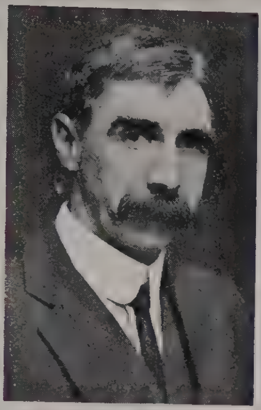

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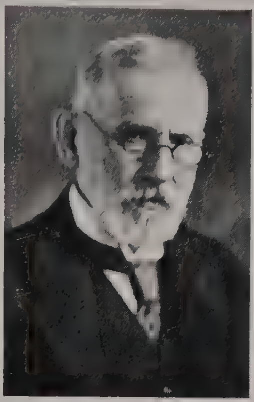

EHRLICH

HEWLETT

CRILE

YAMAGIWA

ITCHIKAWA

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Smith: Fifty Years of Pathology

LAFONT

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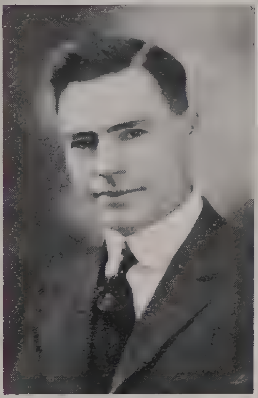
HAWKINS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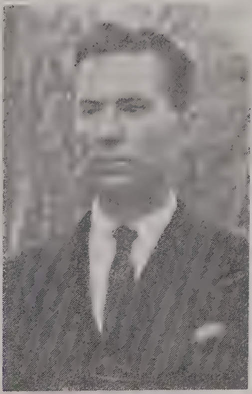
WOLF

RAND

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WOOD (F.C.)

CULLEN

MAYO (W.J.)

DICK (Mrs.)

DICK

BESREDKA

CALMETTE

BANTING

BEST

SMITH: FIFTY YEARS OF PATHOLOGY

MUIR

POWER

HISS

GOLDBERGER

ANDERSON (J.F.)

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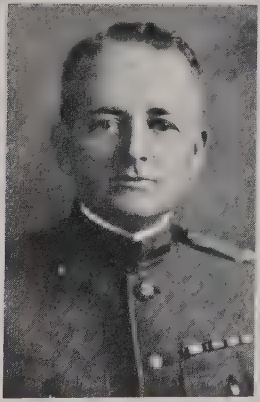
WILDER


SILER

HITCHENS

SMITH: FIFTY YEARS OF PATHOLOGY

SMITH: FIFTY YEARS OF PATHOLOGY

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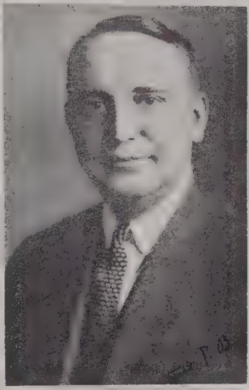
MacCALLUM (W. G.)

ROSENOW

SIMPSON

BLUMENTHAL


BURROWS

FISCHER (ALB.)

FIBIGER

JENSEN (C.O.)

SMITH: FIFTY YEARS OF PATHOLOGY

SMITH: FIFTY YEARS OF PATHOLOGY

HEDGES

BROWN

McCULLOUGH

BRYAN

QUIRK

HASSE

PATTERSON

CHARLES

ELLIOTT

SMITH: FIFTY YEARS OF PATHOLOGY

The group of botanists who planned and originated the Bureau of Plant Industry: Swingle, Waite, Carleton, Woods, Fairchild, Dorsett, Galloway, Smith.

H. T. Ricketts who in 1909 discovered *Rickettsia*, the cause of the Spotted Fever of the Rocky Mountains, and who lost his life in Mexico in 1910.

SMITH: FIFTY YEARS OF PATHOLOGY

NATURE AND AGRICULTURE IN DRY REGIONS OF THE UNION OF SOVIET SOCIALISTIC REPUBLICS[1]

N. M. TULAIKOV
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Definition of the concept "dry region." From the economic viewpoint the term "dry region" is applied to those territories in the U. S. S. R. where agriculture is very unstable, being frequently endangered by extremities of climatic conditions, among which high temperature and deficiency of atmospheric precipitation are more or less common factors. Peculiar to these sections, from the climatological viewpoint, is the predominance of evaporation over the amount of precipitation, with an attendant lowering of relative humidity and a very high total sum of summer temperatures. These regions are usually characterized by light-colored soils—chestnut-colored or fawn-colored semi-desert soils, with a small amount of humus, not infrequently with sharply compacted horizons at a short distance from the surface and with a conspicuous quantity of soluble salts.

Boundaries and area of the dry region.—The dry territory begins as a narrow strip at the western boundary of the U. S. S. R., along the shore of the Black Sea. Widening gradually toward the northeast it practically borders the black-soil area, extending over what is known among the Russian soil experts as southern black soil, and over the chestnut-colored soil, the brown and light-gray semi-desert soil south of the black-soil area. The northern boundary of the dry region runs somewhat south of Kharkov, Ponza, a little north of Samara, through Ufa, and passes eastward into Siberia north of Cheliabinsk, Petropavlovsk and Omsk, almost to the border of China. The entire territory of the U. S. S. R. south of this northern boundary line manifests very clearly the character of an arid zone, stretching up to and including the foothills of Tian Shan, Pamir Alai, Hindu Kush and Kopet Dag, comprising the southern state boundary of the Union.

Roughly speaking, the dry territory occupies an area of approximately 5.4 million square kilometers, which constitutes about 25 per cent of the whole territory of the Union of Soviet Republics.

Natural conditions of the dry region.—Climate and soil are the two most interesting and most important natural factors from the viewpoint of the organization of agricultural industry. It is obvious that these two factors cannot be constant over this immense territory. Differences in latitude, longitude, and altitude of the different parts of the area make it necessary to separate certain

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 17, 1926.

portions of it, which are distinguished by different soil, climate, and methods of agriculture.

An insignificant amount of precipitation (under 40 centimeters annually) and the predominance of evaporation induce a trend in soil processes which brings about its enrichment with soluble salts resulting from the disintegration of soil minerals and the decomposition of organic matter. A characteristic peculiarity of the soils of the dry region is their alkaline reaction and their salinity at a certain depth. A small quantity of humus and the absence of any degree of stable structure of soil horizons is another constant character of the soils of the dry region. These soils are in general rich in nutrient substances and usually require no fertilizers. When an adequate amount of moisture is present—either

FIG. 1. The dry region of the U. S. S. R. Scale, 1:126,000.

through a favorable precipitation or through artificial irrigation—large yields from all crops obtain. Under reverse conditions yields fall to zero.

The most important meteorological factor is the atmospheric precipitation, the amount of which fluctuates within very wide limits within the territory of the dry region. Forms of agricultural utilization of the territory are being evolved in accordance with this distribution of precipitation. Agronomy prevails where annual precipitation is no less than 25 centimeters, while in sections having precipitation under these limits, animal husbandry predominates. There is also in existence an intermediary form, in which both agronomy and animal husbandry play a more or less equal rôle, and the income from farming is derived from both sources.

The seasonal distribution of precipitation, which plays a very important part in the development of various forms of agriculture, presents a characteristic

peculiarity. Summer and fall precipitation is predominant on the plains, occupying a large area of the dry region, while winter precipitation prevails in the mountainous parts. The variability of precipitation is very peculiar to the level dry region: the rainfall is not uniformly distributed during the vegetative period and frequently there are very prolonged periods of drought, which destroy cultivated as well as wild vegetation. Instances in which no rain falls at the beginning of the vegetative period, with the result that the cultivated vegetation perishes, are by no means rare.

What is the nature of the drought phenomenon?—In the years of drought the agricultural part of the dry region is characterized not only by an inadequate quantity of soil moisture, but also by a pronounced deficiency in air humidity. Because of low relative humidity plants transpire large quantities of water. Due to the scarcity of this water in the soil, cultivated plants suffer at first, and later die, unless a timely rain arrives. In this respect, particularly susceptible are the grains—wheat, oats, barley—which require for their development a large amount of moisture at the beginning of growth. Much more resistant are tillable plants which require very little moisture for their early growth and utilize well the moisture of the second part of the summer, which is hardly used at all by spring plants.

Why is the agricultural part of the dry region so frequently afflicted with crop failures?—In pre-war times, as well as now, the agriculture of the dry region was and is characterized by the predominance of spring crops, principally wheat. In the territory of the lower Volga, according to the census of 1916, 55 per cent of the total cultivated area was occupied by spring wheat. All grains occupied 93 per cent of this area. Since, however, under the conditions of primitive agricultural methods grain crops are particularly dependent upon moisture at the beginning of their growth, they usually perish in a case of lack of rain in May, which is the most constant month in this respect. The amount of spring precipitation determines also the quantity of forage on natural meadows. Therefore, in the years of spring drought both spring crops and natural pastures suffer. Stock perishes, along with crops, and the agriculture faces a crisis if no reserves of grain and feed are on hand.

Is there a way out of the existing situation?—As a result of the work conducted by experimental institutions in the region of dry agriculture of the U. S. S. R., it has been established with a definite certainty of success that there may be a number of measures in the domain of technique and management which would show the agricultural population a way out of the present critical situation. This experimental work has revealed that the yield of winter crops may be almost tripled, but only through the introduction of better, improved methods of agriculture, perfected in accordance with the natural conditions of the region. The yield of spring crops may be doubled. Increased yields of winter crops may be practically guaranteed, though no such guarantee may be assumed in regard to early spring crops.

In order to utilize the precipitation of the latter part of the summer and of the autumn, it is important to introduce into the system of agriculture plants from

the tillable group, since they develop mainly during the second part of the summer. The number of these plants is quite large and varied. Different plants of this group may fit different conditions of the agricultural part of the dry region. Among them are plants of industrial and technical nature, and plants which may be used as fodder for animals. To this group belong: sunflower (oily), mustard, corn, sorghum, millet, carrots, beets, potatoes, watermelons, melons, and pumpkins.

For the purpose of creating a reserve of fodder, experimental institutions have well worked out the requirements of the culture of forage crops—including alfalfa, brome grass, and a number of annual forage plants.

Aside from working out the proper methods of cultivation of these plants, an enormous amount of work has been done by experiment stations of the dry region with breeding new varieties, more resistant and better adapted to the severe natural conditions. A particularly extensive work along these lines has been carried on with spring wheats, sunflower, forage crops, and winter wheat. The use of the selected material on a large scale increases the productivity of agriculture to a high degree.

The importance of stock raising in the agricultural part of the region.—It is only in those sections of the region where annual precipitation approaches 40 centimeters that the agriculture is more or less stable and is not bound very closely with animal husbandry. On the other hand, the organizational unity of agronomy and animal husbandry manifests itself very clearly in other large sections of the region where stable farming may be built up only on the complete unity of these two branches. The insurance crops in a field culture—the tillable plants—are for the most part forage crops. They have no satisfactory market and must be worked over on the farm through the medium of animals, and thus transformed into more readily transportable products, such as meat, fat, milk, butter, wool, hides, etc.

If weather conditions are such that they present a menace to the valuable spring crops (wheats), there may be still a good harvest of tillable crops which may furnish either a marketable product, as sunflower, or a large quantity of fodder. This latter, transformed by animals, will merely shift the main source of income from agronomy to animal husbandry, and the agricultural economy will face ruin, as is the case with typical grain farming.

In those parts of the dry region where agriculture is still less safeguarded by precipitation, stock raising is the only form of agriculture which insures utilization of meager natural resources. In such sections, it molds itself into forms of nomadic farming when the fodder requirements of the stock can be satisfied only by a system of moving from one place to another. The agriculture in such cases has a primitive form, with an incidental sowing of relatively drought resistant plants, such as millet, which in some parts of the dry region is the only cultivated plant.

Governmental measures for the reconstruction of agriculture in the dry region.—Continued bad harvests in the dry region of the U. S. S. R. have always required considerable aid on the part of the state in the years of failures, although in

favorable years this region has been the source of enormous quantities of export wheat. The czarist government waged a fight only with the consequences of bad harvests and not with their causes. The ruin of agriculture on the Volga River, caused by war, revolution, and a series of unfavorable seasons within the recent period of time, induced the Soviet Government to give special attention to this vast region, and it has been decided to make this study the order of the day.

A detailed plan of reorganization of the whole local agriculture has been worked out in accordance with the accumulated findings of experiment stations and the experience of more progressive economies. This plan fundamentally aims at the creation of a system of agriculture fully in harmony with modern scientific data. To accomplish this impressive program of reorganization, embracing tens of millions of hectars, the Soviet Government granted a credit of 77 million roubles (for three years), which is to be used for a complete reorganization of economies of individual village associations, communes, artels, and other cooperatives. The receipt of the credit for this reorganization is connected with the employment of resources of the reorganizing economies. In order to make this work more productive and of greater interest to the population, economies desiring to obtain the credit and to effect reorganization form separate nuclei distributed over the entire dry region that was afflicted by the bad harvest in 1924 (along the Volga River and in North Caucasus). These nuclei of reorganized economies are to serve as models of the new organization of agriculture for the surrounding population.

Along with the reorganization of the agriculture itself, the government finances the establishment of markets for the resulting agricultural products, and the improvement of transportation and storage of these products. At the present time a construction of bacon factories, stock yards, and cold storage plants is under way, and the work of mills for plant products (oil plants) is being broadened.

This work has already been started, and during the winter of 1925-26 plans have been perfected for the reorganization of separate collective units in various governments on the Volga River which are to be put into effect during this season.

All scientific and experimental institutions, with their entire personnel, have been drawn into the organizational and practical work of this undertaking.

SOIL ACIDITY, PLANT GROWTH, AND ITS PRACTICAL APPLICATION[1]

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Even in ancient agriculture the difference between the properties of acid and alkaline soils was observed. It was also known that as a remedy for acid soils one could use lime. By the experience of early agriculturists our farmers also know where one crop grows best and another fails, and where lime may be used. One might imagine, therefore, that there is nothing more to be done in regard to the lime question. On the contrary, one may then answer that the experience won by the farmer is paid for very dearly. There have been years and years of low crops and of loss between the early times and the present.

Agricultural science and the work carried out in this branch of learning are supposed to explain the data gained by experience and to show the reasons why plants yield much or nothing. It seems to me as if we have come now so far in our knowledge of the soil acidity question that we are able to help the farmers in their work.

In a series of pot experiments I found that the different cultivated plants behave in different ways towards the soil reaction. On an acid medium (about pH 5) rye, oats, potatoes, swedes, and timothy grass thrive best; at a pH of about 6 clover and turnips do well. On a neutral or weakly alkaline soil wheat, sugar beets, barley, and alfalfa reach their optimal yield.

Examining about 500–700 Swedish farms in different parts of the country with regard to the soil reaction, I found that on the acid farms the above-mentioned acid-loving plants yielded best and here the neutral-soil plants gave low yields or no yields. On the other hand, on the neutral farms the acidophile plants gave little or no yield, while the neutral-soil plants yielded most satisfactorily.

With regard to the sugar-beet, the Swedish Sugar Co. started very extensive work under the supervision of the author. More than 250,000 soil samples, from every hectare of land grown with sugar beets during the last three years, have been examined. When comparing the results from the soil survey with the yield, one finds that the sugar beet has its optimal growth at a pH between 7.0 and 7.5; exactly the same thing was found in the pot experiments.

For barley and tobacco, similar investigations have been carried out but on a smaller scale, and the result was that both plants yielded best at a neutral or weakly alkaline reaction.

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 17, 1926.

All these investigations point in the same direction, that is, that our cultivated plants are very different in their requirements regarding the soil reaction. One may utilize this discovery in two different ways; either one may use the plants that are best fitted for the soil, or otherwise one must change the soil reaction to fit the plants.

Different soils need different amounts of lime but by a simple method (titrating the potential acidity with equivalent amounts of calcium hydroxide and calcium bicarbonate) one is able to estimate the amount of lime needed for changing the reaction to a certain point. Rough estimates may be obtained by using the different soil types as a standard. As the examination of the soil reaction is very easily and inexpensively made when using indicators (a test may be made for a few cents) one is able to use this method on a large scale. For industries interested in agriculture, such as the sugar industry, the breweries, the tobacco factories, the flour mills, etc., it would be of great value to examine the soils producing their raw products, in order to have a better control on both quantity and quality. For farmers' associations, agricultural societies, and experiment stations it would be a worthy and valuable work to examine the soils of the farms, giving the farmers advice regarding the crops to be grown on their land and regarding the liming, thus enabling them to earn more on the same area, accordingly enriching the nation and the world.

THE PLACE OF STATISTICS IN THE INTERPRETATION OF EXPERIMENTAL RESULTS[1]

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We are assembled to have a round-table discussion on a most important subject. There are no set papers on the program, but by way of introduction I have chosen to make the following brief remarks:

With the advancement in any field of knowledge and with the attendant accumulation of facts, it becomes evident that the interpretations based on these facts should be soundly and carefully made. The field of agriculture, the facts of which are largely based on experimentation, is no exception to this rule. It is also evident that with the advancement in any field there is, or should be, greater interest and consideration given to the study of the proper methods for conducting experiments. In many fields of agricultural endeavor some of the most important research to be conducted is that having to do with the methods of conducting research. That is, we must conduct experiments to learn how to experiment. Every investigator should be interested in using the best methods for conducting his experiments, as well as the most refined methods for their interpretation.

From the early days of agricultural experimentation to the present, there has been considerable improvement in methods, yet much remains to be done. Along with the newer refinement of method has come the development of statistics and their use in the interpretation of results.

One of the effects of this has been to influence both the methods for laying out experiments and also those used in drawing conclusions from the work. In the early days of agriculture many conclusions were based on general observation and many more upon the results of a single test or experiment. While this is not entirely a thing of the past, it is passing. To those who based conclusions on single tests there never occurred the idea of variability or probability. There are still a few such investigators at the present time. To these investigators the gain due to a certain treatment or manipulation is thought of as due to the difference in treatment, and their interpretations are based on this assumption.

One of the important contributions of statistical enquiry for the experimentalist has been the experimental-error concept. The introduction of this idea has emphasized the fact that when dealing with plat yields or other measure-

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy (Horticulture, Genetics and Physiology participating), round-table discussion, Ithaca, New York, Aug. 17, 1926. Paper 151, Department of Plant Breeding, Cornell University, Ithaca, New York.

ments of similar kind, we are dealing with a variable. That is, when we say that the yield of a plat is 40 bushels, we do not mean to say that this is an absolute value. Under the conditions of conducting and measuring an experiment we obtain results that lead to an expression of 40 bushels. However, if it were possible to repeat this experiment under the same identical conditions one would be surprised if he obtained just 40 bushels.

What has been the effect of this on experimental work? It has shown how useless it is to base conclusions on the results of single tests and to say that a certain gain, or loss, from a treatment applied to a single plat is all due to treatment. It has shown that all experiments are affected by many factors which produce variability in our results. One of these very important factors for those conducting field experiments is soil variability. While formerly we were in the habit of stating that a uniform piece of land was selected for a certain test, we now, except for those few who still may think otherwise, recognize that there is no such thing as a uniform field. One need only to conduct an experiment on a field the most uniform in appearance to learn that uniformity does not exist. To be sure, some fields are more variable than others, but it is impossible to find a field that is absolutely uniform.

The result of this was that investigators began to replicate tests and multiply observations. The yields or other results were then expressed as the average of all the tests. In many cases these averages were used and discussed as though they were definite exact values and not subject to variability or experimental error at all. Such methods, however, have been called in question and criticized. This led to a more general application of the experimental error idea. Many investigators began to calculate probable errors for their results and this led to the question of what precise methods to use.

The first more exact methods that were used were those developed for astronomical and other similar measurements. These methods are known as Bessel's and Peters' methods for calculating probable errors. Sometimes the usual methods of calculating the probable error of a mean from the standard deviation of the series (probable error of the mean $v = \pm 0.6745 \frac{S.D.}{\sqrt{n}}$[2] was used, but for a small number of observations this method does not give the best measure of the errors. This method of Bessel and of Peters differs from the others in that in the former a correction is made for a small number of observations, such that errors will be larger than when the usual method is used. This is done by substituting $n-1$ for n. For any number of determinations or observations beyond 25 or 30, there is little difference between the results as obtained by Bessel or Peters and the usual method. The use of these methods was criticized by some in that they were not applicable to small numbers. In many instances it was clear that where only 4 or 5 observations had been made, the distribution of the errors was not normal. In such cases the probable errors, as calculated, are not reliable in themselves, but show that the data are so variable that it is unwise to place much dependence upon the results.

[2] In this formula S. D. refers to the standard deviation and n to the number of observations.

The question may be raised, however, as to whether much reliance may be placed in any constant or average that is based on a few observations. It seems only fair that even with small numbers it may be proper to calculate the probable error merely as a safeguard in drawing conclusions. While, as has been stated by Brunt,[3] calculations of errors should not be based on small numbers, and from a mathematical standpoint this is true; nevertheless, in practice it seems that the calculation of errors even for a small number of observations will be very useful. Such errors will indicate the variability of the material with which one is working and should make one more cautious in his deductions.

It may be stated that when one is using small numbers it is advisable to use the corrective terms suggested by Pearson[4] for correcting the standard deviation for small samples. The effect is to render larger probable errors and therefore the conclusions drawn from the results obtained will be more reliable.

One of the objections to these methods as usually applied to plat observations, or observations taken in different years, is that when deviations are figured from the mean of the series the probable error is rendered larger, since the systematic error, or errors, due to season and the like is contained in the final value. Then when two results are compared and the probable error of the difference is obtained, this error is apt to be too large, since there may be correlation between the results. In such cases it has been pointed out by several writers that the full formula for the probable error of the difference should be used. This well-known formula is

$$P.E._{A-B} = \pm 0.6745 \frac{\sigma_{A-B}}{\sqrt{n}} \quad \text{when}$$

$$\sigma_{A-B} = \sqrt{\sigma_A^2 + \sigma_B^2 - 2r_{AB}\,\sigma_A\,\sigma_B}$$

This takes the correlation into account, but requires more calculation. A short method was suggested but not elaborated by the present author.[5] Apparently, however, this has been overlooked by most of the writers offering the above suggestion. This consists of first obtaining the difference between *A* and *B* and then calculating the probable error by Bessel's method. This gives the same results as the longer method of calculation and is much more readily obtained. The chief objection to this method is this: unless a correction is made for small numbers, the odds obtained will be high, and one is apt to be misled into a "false feeling of security" as Student[6] states. If, however, the proper correction, as suggested by Pearson and mentioned earlier, is made, the odds obtained will be lower and more reliable. Student has made a very important contribution to the subject and has presented a table which may be used in interpreting the results obtained from small samples. By the use of his table,

[3] Brunt, D. The combination of observations, Cambridge, 1917.

[4] Pearson, Karl. On the distribution of the standard deviations of small samples. Biometrika **10**: 522–529. 1915.

[5] Love, H. H. The importance of the probable error concept in the interpretation of experimental results. Journal of the American Society of Agronomy, **16**, No. 6: June, 1923.

[6] Student. The probable error of a mean. Biometrika. **6**: 1–25. 1908.

or the modification given by Love,[7] it is possible to form some judgment as to the reliability of results when the nature of the material has been such as to render it impossible to make observations on a large number of individuals.[8] Student's table for the interpretation of results is the best one available to us at the present time, and is used whenever it is possible.

While some investigators have been using statistical interpretation for some time, the subject as a whole is comparatively new to a great majority of workers. This fact, coupled with the further truth that comparatively few investigators have had any special training along these lines, means that there is danger of misapplication and misinterpretation.

What are some of these dangers? In the first place the wrong method for analysis may be chosen. This may lead, as has been pointed out in earlier papers, to a tendency to discredit or discard the method and go back to the less reliable methods of earlier days. Another error that is likely to be made is that of comparing results that for various reasons cannot be directly compared, such as, for example, plats that are too far apart, and other similar cases. Such examples have been used to attempt to show the limitation of a method when it is not a case of the method being limited at all. We do not discredit the method of addition if one attempts to add bushels, pounds, and gallons together and to express the result as pounds. No, the limitation should not be applied to the method but rather to the one who makes the wrong application.

Another point of danger is where an investigator has applied some method as, for example, Student's method of interpretation and found, say, a difference of 5 bushels with odds of 100:1 and then leaves the impression that all this is due to the difference in treatment, variety, or what not. This must not be done. The method does not tell what caused the difference but merely indicates there is or is not a significant difference. While experimenters have been doing this for years before the day of statistical interpretation, it should not longer be done. This kind of error has been used to show the limitation of method. While it is amusing that it has been so used, this again is not a fault of the method used. It rather points to the danger of making certain calculations and then placing too much confidence in the final result. At no point in the operation does statistical analysis do away with the need for common sense.

[7] Love, H. H. A modification of Student's table for use in interpreting experimental results. Journal of the American Society of Agronomy, **16**, No. 1: January, 1924.

[8] Since writing the above a copy of Student's paper, "Mathematics and Agronomy," Jour. Am. Soc. Agron. **18**, No. 8, has come to hand and is recommended to all interested in the subject.

THE BACTEROID-LIKE FORM AND IMMUNITY IN LEGUMINOUS PLANTS[1]

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Having elaborated his investigation on the subject, "Reaction immunity in the root tubercles of legumes" (Annali di Botanica di Roma, 1924) reference is now made to several results on the question of the bacteroid-like form assumed by the organisms affecting the roots of the leguminous plants. The experiments were made primarily with the garden pea (*Pisum*) in which plant the bacteroid-like forms are most conspicuous. From the tubercles of this plant there were extracted by pressure some antibodies of the type of the agglutinin agent, as exhibited by appropriate agglutination tests arranged in vitro. The cytological study of the tubercles showed that the nucleus of the infected parenchyma cells, immediately after the entrance of the germs into the cell lumen (plasma), undergoes a noteworthy hypertrophy, accompanied by a multiplication of the number of nucleoli, and by a denser organization of the nucleoplasm, with an increase in the amount of chromatin. Even the cell volume increases notably.

The causal organisms, that always possess the form of small rods in the first phases of penetration, begin to assume (after the above noted reaction of the host cell) a special or enlarged aspect that may be called the phase of preparation for the bacteroid-like form. From this it passes rapidly to the true bacteroid form with cells either of the type of *x* and *y* or arborescent. The appearance of bacteroid-like forms is accompanied, moreover, by the appearance, in the tubercles, of *antibodies which may be found in vitro.*

These investigations have been extended to other plants and reference will be made to the results obtained with the genus *Phaseolus*, in which there are *never* bacteroid-like forms in the tubercles—not even in the more advanced phases of tubercle development. In this plant the nucleus of the invaded parenchyma cell does not undergo those hypertrophies, attended by an increase in the density of the chromatin, peculiar to the pea, but instead the nucleus becomes a little larger—often loses the nucleoli—and development ceases at a reticular stage, poor in chromatin.

Of the juices extracted from the root tubercles of the kidney-bean, it has not been possible thus far to recognize antibodies of a specific or nonspecific type in any phase of the vegetative development of the plant. Placing in relation, then, the time of appearance of the antibodies with the simultaneous appearance of the bacteroid-like forms, it follows that the latter should be considered the

[1] Presented by title before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 17, 1926.

true reaction forms of the organisms, produced in response to the action of specific antibodies, the presence of which is possible only if the host cell has reacted intensely. The case of the kidney bean, with the weakest reaction, is not accompanied by bacteroid-like forms, but by rod-like forms. The kidney bean is not, moreover, an isolated case in this sense, *Robinia* and other plants being probably related to the kidney bean type.

The cytological examination of the organism in the form of bacteroids always reveals a thickening at each extremity of the branches. This is strongly chromophile and conforms in respect to staining to the nuclear type, behaving as true masses of chromatin. This fact is interpreted as a corroboration of the humoral reaction in the sense that the germ defends itself against the antibodies produced by the host through magnifying the activity of its nuclear masses; analogously to what is proved by the cells of the host in respect to the parasite. *The bacteroid-like form is therefore a reaction form, a result of the immunity processes called forth in the plant.* This form has for the most part lost the capacity of reproduction and in some cases it has the tendency to transform itself into forms similar to spore-bearing cells.

RUSSIAN FIELD CROPS IN THE UNITED STATES*

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Introduction

The phenomenally rapid development of the material resources of the United States of America is in no small measure due to the influx of the best and most enterprising laborers of the world, who unhampered by tradition, routine and bondage, were able to develop the genius of initiative and free competition—two of the most important factors leading to progress. Immigration has for two centuries been pouring fresh strength into the United States, and representatives of nations of the entire world have been constantly assisting in erecting the miraculous edifices of the fairy-like Republic—the land of skyscrapers.

About three quarters of a century ago, the first nameless, unknown and uninteresting Russian immigrants came to America in the quest of truth, justice, free life, and free labor in a new land across the ocean. They belonged to the religious sects of Mennonites, Molokans, Dukhobors, Baptists and others. Scouts sent out in advance had already been all over the States in their search for a new fatherland. The native of Central Russia was looking for his "Ivanovka" (his native village), where building lumber would be at hand, with water nearby, where rye and wheat could be raised and his log cabin built. "Ivanovka" was found in Saskatchewan, in Alberta, in British Columbia, in Minnesota, and in California; here the pioneers were principally Molokans and Dukhobors.

Natives of Southern Russia and Ukraine were looking for the freedom of their Steppes, for virgin soil, where wheat could be grown, also "proso" (millet), flax, and sunflower. They found their "Kieff" in North and South Dakotas and in Manitoba, Canada.

The Crimean Mennonites, who originally came from Prussia in 1770, but who in the course of time became completely identified with Russian life, including the communal mode of landholding, settled in Kansas, Nebraska, South Dakota, and Washington, carrying away with them the memory of Crimea, with its luxurious colonies—Molochnaya and others.

Many Molokans settled in Arizona and California, also in Mexico, as these parts resembled their Caucasian homelands.

The dark-gray mass of Russian immigrants, walking down the gang-planks to the pier after a long trip across the ocean, carried heavy unwieldy sacks filled with all manner of things full of memories, and at the bottom of each of these

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sacks was sure to be found a bundle containing precious seeds of plants grown back home.

The Mennonite from Molochnaya and other colonies of Crimea and the Ekaterinoslav province brought with him Crimean wheat, Turkey Red, Kharkov wheats, Kherson oats, and other seeds. The Molokans brought Sandomirka and Kustavka wheats. The natives of Kieff brought flax, sunflower, "Proso" (millet) and wheat, and still in another little bundle the seed of watermelons, melons, and of Russian flowers. And now, the little bundles of seeds carried away from home were carefully unknotted, and calloused horny hands threw the Russian seeds into the soil of the New World.

Years passed. Persevering labor had met and overcome the first failures. Acclimatized to the new conditions, no longer a serf of the Russian land baron, not a peasant, or "moujik"—but a Russian of the future, the free farmer began to harvest enormous crops, and the arid prairie, the equivalent of the Russian steppe—the Russian tchernozem—revived, became covered with a golden harvest of Russian wheat, or other Russian crop, and a ceaseless stream of grain began to flow to the elevators, the mills, to the centers of the grain country—Minneapolis, Kansas City, Omaha, Chicago, St. Louis.

The first crops raised by the early settlers in this country—in the moist, warm climate of the East—were wheat, oats, and rye obtained from England, France, and other countries with similar climates. A hundred years passed, and the first American pioneers began to move westward, where they found the prairie dry and inhospitable and everywhere land seemingly too poor for an immigrant coming from the moist East or the Corn Belt. Consequently, for a number of years cattle breeding was the principal occupation in the Great Plains, diversified agriculture being so far entirely beyond reach.

Meantime, the Russian farmer, seeking his "life zone," brought along with him plant immigrants for this "life zone": drought-resistant varieties of wheat, flax, sunflower, oats, and others. Then, having found a suitable climate in the area of the Great Plains, he was provided with the necessaries for agriculture in that part of the country.

The observant eye of the American agriculturist finally noted among the heterogeneous homesteads of Norwegians, Irish, etc., the modest Russian farms and luxurious Russian flax, wheat, oats and sunflower. Neighbors confirmed the presence of Russian varieties, and the fame of these was widespread—from Manitoba and North Dakota to Minnesota and Kansas.

The Russian innovations at first, however, met with many obstacles: the mill refused to accept Russian wheat for milling as being too hard and the flour too dark; they preferred the snow-white flour. The wheat fell in price and in the majority of cases was rejected. The same fate met spring wheats—Fife and Bluestem—until the year 1870, when with the first wave of Russian winter wheat, Delacroix built a rolling mill in Minneapolis.

Several decades passed and history repeated itself with a second wave of Russian winter wheat from Kansas and Nebraska: Turkey Red, Kharkov, Crimean, etc. They were again refused acceptance at the mills; the flour was

said not to have the correct color, and declared suitable only for macaroni and breakfast foods; the prices fell. This struggle, of which Minneapolis is still the center, continues practically to this day, with the third wave of Russian wheats, Kubanka and Arnautka; but whatever the outcome, Russian spring and winter wheats have already won their place in America and have shifted the center of wheat production to the Great Plains region.

Around this third wave of Russian wheat, Kubanka and Arnautka, a veritable battle has been raging. Accusations were leveled at Hon. James Wilson (then Secretary of Agriculture), for favoring and protecting the interests of the Minneapolis milling trusts, which were lowering prices of the best Durum wheats. Thereupon the Federal Government stepped in to put an end to this feud. The late Dr. Mark Alfred Carleton deserves great credit for a most serious study of this question. However, up to the present time, a difference in the market price still exists.

The above is mentioned by the author exclusively with the view of pointing out the public interest aroused by three waves of Russian varieties of wheat, the demand for which increased enormously during the World War, when the closing of the Dardanelles cut off Russian wheat from Europe; and the United States, together with the Argentine and Canada, were called upon to fill up the gap caused by Russia's absence from the European markets.

The Russian hard wheats have entirely changed the agricultural situation of the Great Plains and shifted the center of wheat production in the United States to the West.

I have previously dwelt upon accidental importations of Russian seeds by immigrants who came from Russia and the attention bestowed upon these seeds by American agriculturists and state officials. This was followed by a period of "fashion" in Russian seeds, when a number of expeditions were sent to Russia for the purpose of importing Russian seeds on a larger scale than heretofore. Plant immigration was organized with the collaboration of a number of agricultural explorers commissioned to Russia by the Federal Government. As a result of these expeditions an enormous quantity of seed material for the Great Plains and arid regions of the United States was obtained. These expeditions brought back Kubanka wheat, Russian varieties of alfalfa, as well as wild *Medicago falcata*, all of which have already undergone selection tests, hybridization, etc., at the experiment stations of various states.

All seeds and plants imported from other countries into the United States since 1897 have been recorded by the United States Department of Agriculture, and those interested in seeing Russia's contribution to the New World in this particular field will be well rewarded by reading the Inventory of Foreign Seeds and Plants, published by the Office of Foreign Seed and Plant Introduction, U. S. Department of Agriculture, where thousands of numbers listed came under the heading "Russia."

Various expeditions considerably increased the volume of Russian seed and plant material imported, but it would be difficult to apportion exactly those amounts which were contributed individually by immigrants, by American

agricultural explorers, or by various Russian agricultural agencies established in the United States.

In conversing with American agriculturists, the author has frequently heard the claim that many of the Russian seeds and plants were not a contribution of immigration, but rather a result of organized importations with the aid of expeditions to Russia, undertaken by individuals. It should be borne in mind that the contribution of the Russian immigrant in respect to plant introduction will never be fully known, for there was no one to record or list his importation. The question as to who imported the seeds first, and who was first to prove that seeds brought from the Black belt of Russia could successfully be adapted to the climatic conditions of the Great Plains still remains unanswered. In any event, a great service was rendered.

In the history of plant immigration we have three distinct periods: (1) Importation of seeds by Russian immigrants for use on soil similar to that of their homeland. (2) Organization of special expeditions to Russia for collecting Russian seeds and plants, undertaken by private individuals (Bernard Warkentin) among the immigrants, and by American agricultural explorers, such as Prof. N. E. Hansen, the late Dr. M. A. Carleton, Frank K. Meyer, Prof. H. L. Bolley, and others. (3) Selection and hybridization of Russian varieties at American Agricultural Experiment Stations, leading to their improvement in various respects and their adaptation to local conditions.

In view of the above differences in the sources of supply it seems quite clear that variations in the nomenclature of some strains of plants are inevitable. However, most of the varieties are listed and it is not difficult to establish their identity.

Each Agricultural Experiment Station conducted its own tests with Russian wheat and each State placed its own special name upon varieties developed, thus making it hard to trace the original Russian wheat through the new name without examining the plant and consulting entries made at the Experiment Stations.

In the matter of colonizing the West and Northwest of this country during the past 50 years, one of the most urgent problems was to find staples suitable for that climate. Plants from eastern states proved unsuccessful owing to insufficient drought and frost-resistance, also lack of adaptability to the rather short vegetation season of the Great Plains. This problem was later solved by the introduction of Russian varieties in the ways indicated.

One of the most interesting facts noted by the writer during his visits to the Experiment Stations in the West and Northwest is that most of them are bestowing considerable attention upon Russian varieties. Still more interesting is the fact (perhaps merely an accident), that experimental fields are to be found in the immediate vicinity of the fields of Russian immigrant farmers.

As a result of selection and hybridization, many introduced varieties have undergone considerable changes and have been improved to such an extent that they now possess special interest to Russians, as desirable varieties for re-importation and re-introduction into Russia—but this is a different story, of

which I shall speak later. Russian staples, such as wheat, oats, alfalfa, etc., may find themselves in the same position as the sunflower, which as a native of America was imported into Russia a century or so ago and was developed under new conditions into an entirely different plant from that of the original wild ancestor (*Helianthus annuus*) in the Northwest of the United States. This product of Russian semi-natural selection came to America, via Novorossiisk, Odessa, and the Baltic ports, and now flourishes in Wisconsin, North Dakota, and Montana under the names of "Russian Giant" and "Russian Mammoth."

In dealing with various field crops of Russian origin the author is endeavoring to give the genealogy of different strains of Russian varieties, without laying claim to a complete description,[1] having at his disposal only that material which he was able to secure during his trip to the Northwest of the United States for the purpose of collecting specimens for the Exhibition held in October, 1921, in New York City, by "America's Making," under the auspices of the City and State of New York.

1. Hard Spring Wheats—Fife and Bluestem

Hard spring wheats, as has been proven by investigations, were first introduced into the Great Plains of the United States and Canada about the middle of the last century, but up to the year 1870 were of no commercial importance, their development being hampered by difficulties of milling without rotary mills. The best known varieties of hard spring wheat are "Fife" and "Bluestem." Fife has a white bare chaff, and Bluestem a white velvety chaff. Both varieties have red kernels and no beards.

Origin of Hard Spring Wheat. It is definitely known that Fife wheat originated in the northern regions of the Volga River valley in Russia, where in the past, and even today, a variety very similar to it, called "Ghirka," is cultivated, as well as a number of other kindred varieties.

At this late date it is difficult, in view of lack of proper records, to state definitely the exact time or circumstances of the first importations of these varieties into the United States. In the "Canadian Agriculturist" of 1861 there appears the following:

> About the year 1842, Mr. David Fife, of the township of Otonabee, Canada West (now Ontario) produced through a friend in Glasgow, Scotland, a quantity of wheat which had been obtained from some steamship cargo direct from Danzig. As it came to hand just before spring seedling time, not knowing whether it was a fall or spring variety, Mr. Fife continued to sow part of it during that spring, and awaited the results. It proved to be a fall wheat, as it never ripened, except for three ears, which grew apparently from single grains. These were preserved, and although sown the next year under more or less unfavorable conditions (quite late in the season and in a shady place), at harvest time it proved to be entirely free from rust, whereas all wheat in the neighborhood was badly rusted. The produce of this was carefully preserved and from it sprang a variety of wheat known all over Canada and the Northern States by the different names of "Fife," "Scotch" and "Glasgow."

[1] A splendid work, "Classification of wheat varieties," by Martin and Clark, appeared after the author wrote this paper, and has therefore not been used as reference. The Russian varieties of wheats listed there are indicated with a fine set of maps, confirming the statements given here.

According to Carleton[2]: "This traces the introduction no farther than Danzig, but it is very probable that the shipment was made from Russia." Absence of this variety in Germany is the best indication of the truth of Mr. Carleton's assertion.

Fife and Bluestem are grown principally in North Dakota, South Dakota, Minnesota, and parts of Wisconsin and Canada. These were the first spring wheats of the hard variety introduced into America. At the present time they are confronted with competition of other varieties, Marquis, which first appeared in Canada (this being a hybrid between Calcutta and Australian Red Fife) and of other Russian varieties—Kubanka and Arnautka.

The era of Fife and Bluestem is apparently drawing to its close—and with it the first wave of plant immigration of hard wheats from Russia into the United States.

2. Russian Hard Winter Wheats

"Turkey" and "Kharkov," sometimes also called "Crimean" and "Malakof," are the principal varieties known in America as hard winter wheat. Their characteristics are: medium-sized head, bearded, with white smooth chaff, and a hard red kernel, a little smaller than the usual winter wheat kernel. There is little or no difference in visible characters between Turkey and Kharkov, but the kernel of the latter appears to be, as a rule, slightly larger and a little darker than that of the former. The gluten content of hard winter wheat is large, about equal to the hard spring wheat, but differs somewhat in quality. "The expansive power or 'strength' is slightly less in hard winter wheat. There is also perhaps a trifle more color in hard winter wheat bread."[3]

Distribution in Russia. Hard winter wheat is grown in Russia in the area north and east of the Black and Caspian Seas; also, in the whole of northern Caucasus. The original area where it was grown apparently included territories of Crimea, Ekaterinoslav, Kharkov, Stavropol, Don, and Kuban. The climatic and soil conditions of this area are very similar to those of the Great Plains region, embracing Kansas, Oklahoma, parts of Texas, Colorado, Nebraska, and a small part of South Dakota.

First Importations into America. Some share in the importation of hard wheats, without any doubt, belongs to the Russian Mennonites. The history of its importation is closely bound with the movement of immigrating Russian Mennonites into the Middle Great Plains region. The Mennonites first came to southern Russia from Prussia in 1770 (during the reign of Empress Catherine II) as previously mentioned. A century later they migrated again, this time to North America, where they settled principally in Kansas and Nebraska. A majority of the immigrants came from the Molochnaya Colony of Crimea, and partly from Ekaterinoslav province. I may quote again from Dr. Carleton[4]:

[2] Carleton, M. A., Hard wheats winning their way. Yearbook, U. S. Dept. of Agr., 1914, p. 393.

[3] Carleton, l.c., p. 348.

[4] Carleton, l.c., p. 399.

The first settlements in Kansas were established in 1873 near Newton, Halstead and Mound-idge. Each family brought over a bushel or more of Crimean wheat for seed, and from this was rown the first crop of Kansas hard winter wheat.

Bernard Warkentin, a miller, who erected mills at Newton and Halstead, was chiefly instru-iental in introducing Turkey wheat, but two other men were associated with this pioneer move-ient of the Mennonites—Christian Krehbiel, then a farmer, who later, in 1886, erected a mill at Ioundridge, and C. B. Schmidt, acting as immigration agent for the Sante Fe railroad.

At the time of introduction of Karkov wheat several lots of Turkey or Crimean wheat were nported by the Department of Agriculture from the *Molochnaya* district of Taurida.

Thus the Mennonites were first to import these varieties. A year later the Kansas Millers Association, through Bernard Warkentin, imported 15,000 bushels of Turkey wheat and it was sold to farmers in Kansas and adjoining tates as seed material, the source of the seed having been recommended to Mr. Warkentin by Dr. Carleton, who wrote (p. 405): "For a time this strain eally appeared to give slightly better results than the usual Kansas seed, but n a few years its influence disappeared."

Later on, the importation of seeding material for these varieties of hard winter vheats, as well as of other Russian varieties, was taken over entirely by the J. S. Department of Agriculture.

A number of expeditions were then undertaken by the explorers previously nentioned and the results are amply illustrated by thousands of registered items n the Inventory of Foreign Seeds and Plants imported by the Office of Seed ınd Plant Introduction.

Kansas. The origin of hard winter wheats in Kansas is thus quite apparent. Crops harvested in that state are principally of Russian varieties of wheat, these being also in a great measure responsible for the increase in the yearly average of vield from 9 million bushels (1869–1878) to 72 million bushels (1899–1908).

Besides the Mennonite settlements above referred to, namely, those at Newton, Halstead, and Moundridge, there are similar colonies also in Rossel, Mariana, and Nicoma. In order to estimate properly the importance of the influx of these immigrant-agriculturists, it should be stated that the number of Russians of German origin, principally Mennonites, who have emigrated to the United States up to 1917 was about 91,000 persons.

Nebraska. In moving from Kansas, the same procedure was repeated. The Russian Mennonites were the first to introduce the varieties with which we are now dealing, having settled around Lincoln, Omaha, and other points. According to the General Federal Census of 1910 and later figures of the population of the State for 1915, the number of Russian immigrants in Nebraska was estimated at 32,692. After the importation of varieties suitable to local climatic conditions the annual average crops increased from 4 million bushels in the years 1869–1878 to 40 millions in 1899–1908.

HUMAN IMMIGRANTS AND CEREAL IMMIGRANTS

In the article previously quoted Carleton says (p. 399):

> There is an interesting feature of this introduction of great crops in the fact that the crop and the people who knew best how to grow it migrated together. The Mennonites had been growing

the wheat in its original home many years, although in the Molochnaya district spring wheat was grown up to 1860, when Turkey wheat was introduced there from the Crimea, and entirely replaced the spring wheat.

Further, he explains (p. 400) the practice whereby moisture was preserved, also the "chernyi par," or black fallow—this being in Taurida province, where the local population included the Mennonites.

It is really a summer tillage and there are 4 cultivations: First—a deep plowing, then 3 lighter operations made by very small gang plows or cultivators at intervals of a month. Therefore, these people, on coming to the Great Plains, were already acquainted with practices of early deep plowing and thorough surface cultivation, not until a quarter of a century later widely advocated in this country in connection with dry farming.

From this statement we see a "double service" rendered by the Mennonites in bringing here good varieties and adopting most suitable methods of cultivation; namely, the black fallow ("chernyi par").

Almost an analogous picture is presented by Russian colonization in South and North Dakotas, and partly in Minnesota and Wisconsin. According to official figures, the population of North Dakota consists of German, Russian, and Scandinavian settlers. South Dakota has a predominating percentage of Scandinavians and Germans. The same applies to Minnesota.

We are not in possession of exact data as to importations of Russian varieties of wheat in these states; furthermore, we are afraid of tiring the reader with too many lists of figures and dates. We will confine ourselves to information obtained during our trip to the Northwest, where authorities on this subject at various Experiment Stations and Agricultural Colleges were unanimous in their praise of the qualities of Russian varieties, including Turkey and Kharkov, as being very suitable winter wheats for the Great Plains region.

This success of Russian wheats is well shown by the comparative yields given in a Wisconsin bulletin[5] about 7 years ago.

RESULT OF SELECTION TESTS AND HYBRIDIZATION OF DIFFERENT VARIETIES OF RUSSIAN HARD WINTER WHEATS IN THE U. S.

Notwithstanding the high quality of Russian hard winter wheats, it was quite natural for agricultural experts at the agricultural experiment stations to strive to improve these valuable crops. We are informed by Carleton, an undaunted crusader in the cause of Russian hard wheats, that these seeds reached experiment stations only after considerable delays. For instance, the Kansas Agricultural Experiment Station did not receive them until 1897, and it took 25 years to achieve final victory for this variety on the American market. In this case, too, there was strong opposition to overcome on the part of mills and the market.

As a result of selection tests carried on at various agricultural experiment stations new varieties derived from Russian wheats were originated under new names, as indicated below.

[5] Delwiche, E. J., and D. Leith. Wheat growing in Wisconsin. Agr. Exp. Sta., Wisconsin Bulletin 305, 1919.

Minnesota. (1) *Minnturkey* 1507. This is a popular hybrid obtained from crossing *Turkey Red* and *Odessa* and it produces the finest flour in Minnesota. (2) *Marduck 1505* is also a derivative of Turkey wheat.

Wisconsin. The following varieties of Russian hard winter wheats were received in 1911 from the Kansas Experiment Station: (1) *Kharkof* 38 (Kansas E. S. 382); (2) *Turkey Red* 37 (Kansas E. S. 570); (3) *Crimean* 40 (Kansas E. S. 762); (4) *Winter Kerson* (?) 64, sent from Ashland, Wisconsin, where it was originated; (5) "*Charkov*" 65/609, of unknown origin; (6) *Spring, hard from Russia* 100/305; (7) *Russian Hybrid* 115–119 received from the Bureau of Plant Industry; (8) *Malakhof* 113 and 114, from the same source; (9) "*Charkoff*" 141 (M. C. 22), received from MacDonald College, Canada; (10) *Kharkof* wheat, Pedigree No. 2—Turkey Red.

This concludes the list of Russian varieties of hard winter wheat in Wisconsin where the climate is apparently too moist for these varieties and where other soft wheats compete successfully with them. Besides the Winter Kerson (?) listed above as No. 64 (or 4) and Kharkof Ped. No. 2, no other varieties were originated.

Standard Turkey Red, Wisconsin Pedigree No. 2 yielded 46.7 bushels. In 5 years Wisconsin Pedigree No. 2 has given an average yield of 33.9 bushels an acre, which establishes it as a high yielding strain.

North Dakota. (1) *Turkey Red*, the most popular variety, not subjected to changes; (2) *Kota.*

South Dakota. (1) *Turkey Red* S. D. 144 (C. I. 3689), the most popular variety of Russian hard winter wheats; (2) *Kanred*, S. D. 1098 (C. I. 5146), received at the Experiment Station, after selection tests, under the name of Kansas Sel. P. 762 and Kansas 2401. The basic material was Russian hard winter wheat Crimean; also, Turkey. (3) *Kharkov* S. D. 191 (C. I. 1442), received from Judith Basin, Montana.

Montana. The arid climate and cold winters of Montana call for cultivation of winter wheat. The most suitable varieties here are those grown on the Kirghiz steppes in Russia, Kubanka, and its derivatives, as well as the Algiers varieties. The hard winter wheats known here are as follows: (1) *Turkey Red*; (2) *Kharkof.* In the eastern half of the plains area of Montana, winter wheat is uncertain because of winter-killing. When the crop survives the winter period it generally produces heavier yields than spring wheat. It matures earlier and for this reason often escapes summer drought.

At the Judith Basin Sub-station winter wheat on corn land gave an average yield of 30 bushels, while the average yield of fallow was 29.6 bushels. The average yield at Huntley has been 22.9 bushels on corn ground and 31.1 bushels on fallow. The preceding refers exclusively to Russian varieties of hard winter wheats, the production of which is possible only in the severe climate of the southern part of Montana. I should mention also (3) *Kanred*, and (4) *Montana* 36, the only variety produced in Montana.

Turkey red is grown extensively in the eastern arid part of the state. In the irrigated parts and in the southeastern corner, where there are summer rainfalls,

other varieties of wheat predominate, namely, Club wheat and Australian varieties. At least, this is so in the southeastern part where wheat culture predominates. However, Red Turkey, which previously (in 1918–1919) represented about 7 per cent of all wheat crops in the state, has lately shown signs of increase in the middle part of the state, the area cultivated under this variety having increased to 12 per cent.

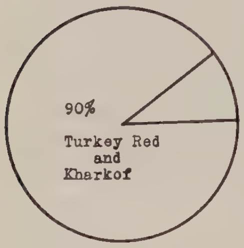

Fig. 1. Ninety per cent of hard winter wheats grown in Montana represents Russian varieties.

The yield of this variety is greater than that of many other wheats, thus at the Washington Agricultural Experiment Station the averages for 1914–1919 are as follows: Turkey Red 326, 37.3; Red Russian 270, 36.6; Winter Bluestem 536, 34.3; Jones Fife 371, 32.9; Marquis 576, 33.6 bushels.[6]

3. Russian Durum Wheats—Kubanka, Arnautka and Others

The hardest of all the above enumerated hard wheats, Kubanka and Arnautka, were the last of the great plant immigrants from Russia to the United States and just like their predecessors, the immigrant hard spring and hard winter wheats were evidently not 100 per cent American and, therefore, met the most stubborn resistance on the part of mill owners and the market. Apparently, they are still not entirely Americanized, in spite of all efforts of those interested.

Characteristics. The Kubanka variety is a bearded wheat with compact flattened heads, yellowish-white chaff and large light-amber kernels, which are extremely hard and "vitreous" in fracture, when of good quality. The gluten content is very large and there is also a large percentage of ash, oil, and sugar.

In Russia, besides Kubanka, there are two other varieties, Pererodok and Arnautka. Sometimes "Beloturka" is used to designate the same thing, though in Russia it is claimed that this variety, when pure, is distinct from Kubanka, having longer and narrower heads.

Original Home. The original home of Kubanka durum wheat is evidently Samara, Saratov, and Orenburg provinces, Uralsk territory, and adjacent portions of the Kirghiz steppe. The best quality comes from the Kirghiz steppe district, where Kubanka is cultivated on virgin soil, with an annual rainfall averaging little more than 12 inches. The center of production is about the middle part of the Samara district and the northern Uralsk territory. Other varieties of durum wheat in Russia are Gharnovka, Velvet Don (Chernouska) and Black Don (Chernokoloska). These were also brought to this country from South Russia, but were soon found to be not quite adaptable to the northern Great Plains.

First Importation of Durum Wheat from Russia. It is difficult to state who was the first to import this variety from Russia. Two facts are known: (1) Before the wholesale importation of Kubanka by Carleton "Durum wheat had been

[6] Schaefer, E. G. (et. al.). Wheat production. Washington, Agr. Exp. Sta., Bull. 159, 1920.

grown in small quantities at scattering points many years before introduction by the Department of Agriculture; commercially it did not exist."[7] It may be assumed with certainty that Russian immigrants who brought along with them hard winter wheats made no exception in the case of Kubanka, the quality of which was undoubtedly known to most of them, if not to all. (2) The expedition of Hansen in 1897–1898 brought durum wheat to the United States from Daghestan (215, 216), Siberia (474, 487), Orenburg (500, 501, 518), Astrakhan (569) and Kharkov (579).

The latest variety from Kharkov is the *Arnautka.* In the Inventory of Foreign Seeds and Plants, U. S. Department of Agriculture, we read:

> Spring variety; one of the best Russian hard wheats. Largely exported to Italy for macaroni. Also much used in Russia for mixing with soft wheats for producing the highest priced flour. It does not make good bread without the admixture of other flour. This variety, which is said to be about the same as Beloturka of the Volga region, is not sent to England, because it sells at a lower price than the softer American and European wheats; but in Italy it commands the highest price, as it is found especially adapted for the manufacture of macaroni. It does best on new land in dry regions and degenerates quickly on unfavorable soil.

This very correct remark belongs to Hansen, who also imported Kubanka from Semirechensk province (858) with the notation "Kubanka for dry lands; the best sort at Vernoe, Turkestan."

Beloturka is also a variety which Hansen was the first to import from Siberia (882) with the notation: "Hard wheat, spring. This has some 'pererodka' or degenerated kernels in it. The idea appears to be that this variety ran out quickly on soils not perfectly adapted to it. This is hard wheat of the Volga region which is shipped to Italy for macaroni and is used for mixing with softer wheats in Russia."

Out of the Semipalatinsk durum wheat (889) Hansen produced the "Tartar," and from the Tashkent (996, 999) he produced the "Chug-bul-dei" with the notation: "A native variety especially adapted for very hot dry regions. Deemed especially promising."

Hansen's example showed the necessity and desirability of importing the splendid Kubanka and its kindred varieties into the United States. In 1899 Carleton made a trip to southern Russia, whence he exported in March of that year Kubanka durum wheat (S. P. I. No. 2953). This proved to be "one of the best durum wheats," which have shown more drought-resistance than our native wheats, and which now yield to the farmers of America over $50,000,000 a year.

We have the figures of subsequent importations of Kubanka from Russia, but these are of lesser interest, inasmuch as the path was already made by previous importations of this excellent plant immigrant.

Distribution by States of Durum Wheats

While the Russian hard spring wheats, Fife and Bluestem, found a home in the moderately dry belt of the United States, corresponding to the central

[7] Carleton, l.c., p. 408.

Russian and black soil belts, hard winter wheats, Turkey Red and Kharkof, occupied considerably more arid regions corresponding to southern Russia.

Kubanka and *Arnautka*—2 drought-resistant strains, enabled agriculture in the Great Plains area to extend further to the West. In addition, Kubanka possesses many other valuable qualities, such as high yields and rust-resistance, which are now attracting the attention of American experts.

We are indebted to Mr. M. Levin of the U. S. Department of Agriculture, for data on the comparative rust-resistance of different varieties of durum wheat:

TABLE I. RUST RESISTANCE OF CERTAIN DURUM WHEATS

Arnautka	C.I. 4072	55%
Kubanka	C.I. 2094	54%
Mindum	C.I. 5296	48%
Spelt	C.I. 6236	52%
Kanred	C.I. 5146	42%
Kota	C.I. 5878	33%
Acme	C.I. 5284	39%

Selection tests made at agricultural experiment stations in the Great Plains produced a number of new varieties of Kubanka and Arnautka, with which varieties and their distribution in the United States we shall deal later on, without making any claim as to the exhaustive nature of our information.

South Dakota. The following characterizes the varieties of Kubanka as distinct from Arnautka varieties known in South Dakota "glumes yellowish white, smooth, flowering glume awned; berries amber colored, large, thick in proportion to their length; sutures narrow, incurving" (Agr. Exp. Sta., Brookings, S. D., Bull. 146). The principal varieties known in South Dakota are as follows: (1) Kubanka S. D. 75 (C. I. 1440), (2) Kubanka S. D. 73 (C. I. 1516), (3) Kubanka S. D. 356 (C. I. 1354), (4) Kubanka S. D. 152 (C. I. 1541).

"Arnautka," or "Goose Wheat," is described as: "Glumes yellowish white, smooth, flowering, glume awned; berries clear amber, large, long in proportion to thickness; suture narrow, incurving." The following are the derivatives: (1) S. D. 148 (C. I. 1494), (2) S. D. 149 (C. I. 1493), (3) S. D. 150, (4) S. D. 151 (C. I. 1547).

The variety that has the greatest distribution is Kubanka, imported by Prof. Hansen, and its derivatives are: "Acme" obtained at the South Dakota Experiment Station in Highmore, and "Monad," a variety obtained from Fargo, North Dakota.

Kubanka and its varieties are grown all over South Dakota, and are concentrated mostly in the Northwest.

The table of comparative yields in the report of H. L. Walster, "Marquis versus Durum wheats" (Circ. 34, Agric. College, N. Dak.) shows the superiority of Kubanka at four state experiment stations.

North Dakota. North Dakota presents a classic example of the rapid increase in yield and profit of Russian Kubanka wheat, as compared with other spring wheats. Two varieties obtained as a result of selection tests carried on principally by Prof. H. L. Bolley at the North Dakota Agricultural College, Fargo, are the

following: (1) *Monad* or *Bolley Wheat D. I.* (C. I. 3320), corresponding to Acme in South Dakota (C. I. 5284); (2) *Pentad D. 5* or *Red Durum* (C. I. 3322).

Those varieties have a good chance to lead where winter wheats are advocated. They are already producing from 5,000 to 10,000 bushels annually and will apparently extend over both Dakotas. North Dakota is at present the arena on which a struggle between Kubanka, Arnautka, and Marquis is going on.

However, the devaluation of hard wheats, which I mentioned above, still remains in force and the 23 cents margin still exists, forcing the farmer to waver when choosing varieties. "Minneapolis demands Marquis as the better milling quality," the author was told by Russian farmers in Kief, North Dakota, "and we have to comply with the demands of the market, as beyond the elevator and the local bank in Kief City we have neither time nor possibility to go without neglecting the farm."

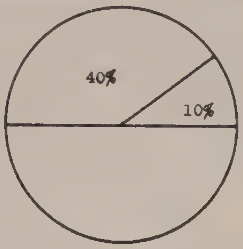

FIG. 2. Forty per cent of the wheat acreage in South Dakota represents Russian varieties of Kubanka, Acme, and Monad; 10 per cent of Turkey Red, Kanred, and Kharkof.

Nevertheless, Kubanka and its derivative Monad D. 5 had gained up to about 45 per cent of the wheat acreage in 1921. The starting point for Monad D. 5 was Stutsman County, whence, through the energetic work of the agricultural agent and help of the Experiment Station at Fargo, Monad D. 5 is pushing forward its gain in acreage.

Minnesota. In this state Kubanka and Arnautka are cultivated on a comparatively small scale. A local variety has been produced under the name of *Mindum* (contraction of "Minnesota Durum"), a result of selection tests with Arnautka.

Montana. Durum wheat is widely known and cultivated as spring wheat in the northwestern part of Montana, the climate of which is similar to the original home of Kubanka—eastern Russia. The Experiment Station at Bozeman recommends this variety as the most suitable for that part of the state. Much attention is devoted to Kubanka and Arnautka at the Experiment Station at Moccasin in Judith Basin, Montana, where the following varieties of durum wheat are under investigation: Arnautka (C. I. 1693), Arnautka (C. I. 1494), Beloturka (C. I. 1520), Pledur (C. I. 1471), Gharnovka (C. I. 1447), Kubanka (C. I. 1440), Taganrog (C. I. 1570), Velvet Don (C. I. 1445), Yellow Gharnovka (C. I. 1444).

Varieties which compete with Russian durum wheats are 2: (1) *Glagalos* (C. I. 2398) (from Transcaucasia) which produced the highest average yield both for the 4-year period (1909–1911) and the 7-year period (1908–1911 and 1913–1915). This wheat is a soft variety and is of less value for milling purposes than the hard wheats. (2) The next highest yielding variety is a durum wheat, the *Pelissier* (C. I. 1584). This variety has a 7-year average yield of 27.2 bushels per acre, which is only 0.4 of a bushel less than the yield of Galgalos. The durum group, as a whole, has yielded a higher average than any other group, but Kubanka, Perorodka, and Yellow Gharnovka are not far behind their competitors.

The states enumerated do not, however, quite exhaust the distribution of Russian durum wheats, which have migrated to the south, to Nebraska and Kansas, and have penetrated into Wyoming, Idaho, Washington, Oregon, and Colorado; the movement cannot apparently be considered arrested, and wherever the precipitation approximates that of eastern Russia and the Kirghiz steppe, Russian durum wheat will be a welcome acquisition to the farmer.

4. Russian Rye

Russian rye is grown in considerable quantity in Michigan, Wisconsin, and Minnesota and is known under different names. It was first used in the state of Michigan, and from there it was distributed among the farmers through various agricultural experiment stations. The rye acreage has increased considerably in Michigan, a fact accounted for by the high quality of Russian rye (see Bull. 105, Michigan Agric. Exper. Station, 1921).

Fig. 3. Percentage of Russian rye in Minnesota.

Of the varieties of rye cultivated in this country, the local "common rye" has the largest distribution; Swedish rye comes next, followed by Russian rye. In the state of Minnesota, for instance, Russian rye represents 20 per cent of the acreage, Swedish rye (Minnesota No. 2) 40 per cent, while the principal acreage is of "common rye."

5. Russian Oats

If we have had difficulty in fixing the exact date of the first importations into this country of Russian wheats, there is still greater difficulty in tracing the first appearance of Russian oats, now representing some of the most popular varieties in the Great Plains. We cannot affirm with full certainty that they were imported by definite groups of Russian immigrants, but we are justified in bringing forward this supposition in the full hope that the near future will produce information to corroborate this statement. The author is basing this expectation on information obtained during his visit to the Northwest in 1921.

A large variety of Russian oats is known here, some of them already having passed selection tests in Russia. Others were imported by the U. S. Department of Agriculture. The author will deal with Russian oats and their qualities in the order of those states which were visited by him.

Wisconsin. Several varieties of Russian oats are grown in Wisconsin, their origin in most cases being definitely known, as they were almost all supplied by the U. S. Department of Agriculture, or by experiment stations of other states. Most of the Russian oats are from southern Russia; some of them from northern and central Russia and from Siberia, these points being their original homes.

While in the case of Russian wheats the starting points of their distribution are Kansas and North Dakota, the starting points for oats are Nebraska and Iowa, which again brings forward the idea of their original importation by

Russian immigrants. Note the following varieties: (1) *Kherson Oats* 49 were received at the Agricultural Experiment Station, Madison, from the Nebraska Station (Lincoln). According to the U. S. Federal Census of 1910, and later state figures for 1915, there were in the vicinity of Lincoln and Omaha 32,692 Russian immigrants, mostly farmers. (2) *Wisconsin Ped.* 7 also originated from Kherson oats after selecrion tests conducted in Wisconsin. This variety has a wide distribution in the southern part of the state. (3) *Wisconsin Ped.* 3, or *Tobolsk Oats*, were sent to the Station by the U. S. Department of Agriculture in 1899 (No. 4341), apparently having been brought from Russia during the expeditions of Hansen or Carleton. (4) *Iowa*, 105, or Kherson Yellow. (5) *Iowa* 103, or Kherson White. Both of those last mentioned were received from Iowa (133 and 134) under the name of Early White. (6) *White Russian* 10 was received from the U. S. Department of Agriculture and was apparently brought from Russia by Carleton. (7) *White Russian* 138.

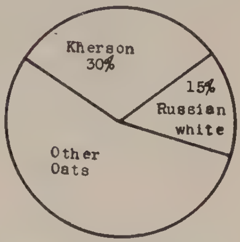

FIG. 4. Russian oats in southern Wisconsin.

These are the records of Russian oats at the Wisconsin Experiment Station. The situation is about the same for the whole of Wisconsin. Nebraska, Iowa, and Illinois were the sources of supply of Russian oats.

Russian varieties of oats occupy the southern part of the state, where early varieties are almost exclusively Kherson oats—one-third of all oat acreage, and constantly on the increase. In the northern part about one-tenth of the acreage is in Russian oats. Figures 5 and 6 show the percentage acreage of Russian oats at the Agricultural Experiment Station in Madison and in southern Wisconsin.

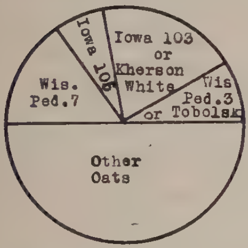

FIG. 5. Russian oats at the Wisconsin Experiment Station.

According to information supplied by Mr. B. P. Leith, of the Agricultural Experiment Station, Kherson oats gained additional laurels in 1921, when the extreme heat and drought attending winds from the Gulf of Mexico wrought havoc with other varieties. The Kherson oats were unaffected and gave a maximum yield.

Minnesota. In Minnesota, Russian oats have a similar distribution and success. The following varieties are known: (1) *Iowa* 2 or ordinary *Kherson*; (2) *White Russian*, being rust-resistant and similar to Kherson; (3) *Ligova* 281 and others; (4) *Swedish Select*, originally from western Europe, brought to America from Russia, having the largest distribution in the southeastern part of the state.

FIG. 6. White Russian oats in northwest Minnesota.

South Dakota. (1) *Sixty-Day* S. D. 165. Sixty-day oats was introduced into the United States from Proskurov, Russia, in 1901, by the United States Depart-

ment of Agriculture. It has become the leading early variety in South Dakota.

(2) *Kherson Oats* S. D. 115. Introduced into Nebraska in 1897 from the Kherson government of Russia, by the Nebraska Experiment Station. On account of its habits of growth the oat is reported as peculiarly adapted to central and western Nebraska. In 1902 in Nebraska, when Swedish Select oats lodged so badly as to make it impossible to determine yield, Kherson oats, though partly lodged, yielded 52 bushels per acre. Sixty-day, partly shelled and yielded only 30 bushels per acre (Nebraska Bulletin 82).

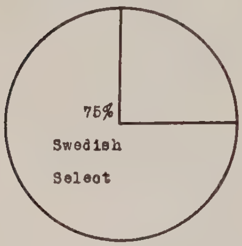

FIG. 7. Swedish select oats in southeastern Minnesota.

(3) *Swedish Select* S. D. 134. This variety was introduced from St. Petersburg, Russia, by the United States Department of Agriculture, Office of Cereal Investigations, in 1899. It originated in Sweden, and was introduced into Russia through Finland. High yields are reported from this oat in many parts of the United States.

(4) *North Finnish Black.* The Bureau of Plant Industry of the United States Department of Agriculture introduced the above named oat into the United States. It was brought from Tornea, Finland, and is a standard variety of that region.

(5) *Belyak* S. D. 336 obtained from Moscow in 1904, having come originally from the Svalof Experiment Station in Sweden. It is botanically the same as the Swedish Select, having been selected at the same experiment station as the former. Belyak and Swedish Select are apparently strains of the same variety.

(6) *Tobolsk.* This variety was introduced in the year 1899, probably by Hansen. It was an original importation from the Tobolsk Province, Russia. During a period of 9 years this variety gave an average yield of 35.9 bushels per acre. The largest yield was 57 bushels per acre in 1901. The smallest yield was 5 bushels in 1907. This variety matured in 103 days; the same length of time was required for Swedish Select to mature. During 1908 this variety yielded 21.8 bushels per acre with 95 per cent stand and tested 25.5 pounds per bushel. This variety grows about 48 inches in height.

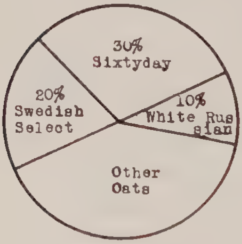

FIG. 8. Russian oats in South Dakota in 1921.

The distribution of Russian oats, which is considerable, is fairly even throughout the states. Of the total acreage of oats, 30 per cent is made up by *Sixty-day* (*Cole*), 20 per cent by *Swedish Select* and about 10 per cent by *White Russian.* The balance is made up by non-Russian varieties.

The yields of Russian varieties of oats are tabulated in Bulletin 110 of the South Dakota Agricultural Experiment Station, Brookings, 1908; also in Bulletin 149 of the same Station. From the former it will be seen that Sixty-Day, Swedish Select, North Finnish Black, and Tobolsk lead in yield, the first mentioned with 62.5 bushels, easily twice the yield of the majority of varieties tested. The long-term tests gave very good

results. It is only necessary to quote some conclusions given in Bulletin 149, referred to above, on the basis of average yields, as follows: The highest average yield of grain has been secured with Sixty-day, an early variety. The second highest yield has been secured with Swedish Select, a medium variety. The third highest yield was secured from North Finnish Black, an early variety of evidently poor color.

North Dakota. Of the 5 varieties of oats cultivated in the northeastern part of North Dakota 4 are Russian strains, namely: (1) Sixty Day, (2) Swedish Select, (3) White Siberian—all 3 early varieties—and (4) White Russian, a late variety. In the southwestern part of the state so-called Mountain oats predominate.

Montana. The oats grown in Montana are exclusively of Russian origin, namely: (1) Kherson (C. I. 459), (2) Sixty-Day (C. I. 165), (3) Sixty-Day Selection (C. I. 789), (4) Sixty-Day Selection (C. I. 625, 626), (5) Swedish Select (C. I. 134), (6) White Russian, (7) White Tartarian, (8) Siberian (C. I. 741). Numbers (6) and (7) are late varieties. Among other varieties are *Victory*, C. I. 742, and *Silver Mine*, C. I. 714.

The question of quality is clearly illustrated in a diagram presented in Bulletin 398, U. S. Department of Agriculture (p. 29).

The main center of production of oats is in those states, the climate of which more closely approaches that of southern Russia and in which Russian oats have a large distribution. Iowa, Kansas, Nebraska, and Illinois are no less acquainted with Russian varieties than the above enumerated states, whose production of oats was under investigation. It should be added that Russian oats found their way to the market without any complications; American horses, too, found them quite palatable. The only exceptions are the dark colored or black oats, which are still not in favor, although in every respect quite satisfactory.

6. Russian Broom Corn Millet (Proso)

Prior to the appearance of Russian immigrants, America did not know this plant as a cereal, which, in addition to being an excellent food for birds also provided a food product for human consumption. There is reason to assume that it was brought here by all Russian immigrants, but more particularly by natives of the Kief and Ekaterinoslav provinces, as well as by the Mennonites. Millet is now raised exclusively by natives of Russia, both for bird seed and for cereal ("Psheno"), that is, prepared for use as porridge. In spite of the undoubtedly good qualities of millet and its high yields, it has so far not become popular in America. Russian farmers in North Dakota are unable to understand why millet is not accepted at the grain elevators; nor is there any market for it, and it is being sold mainly among Russians.

Another obstacle in the way of popularizing millet is the absence of the necessary machinery for handling the millet—"Psheno hullers" and special millet hulling places, which are in existence all over Russia. Not until August, 1917 was any "Proso" huller to be found in this country. Prof. Hansen, in "Northern Novelties" for 1919, writes:

In August, 1917, the first Proso huller in America was received by the South Dakota State College from Russia. I ordered it from Russia, but owing to the submarine campaign, it had to be sent via Siberia and Japan, so it was one year and seven months on the way. This machine was exhibited at the South Dakota State Fair, at Huron, September, 1917. If necessary, this machine may easily be duplicated, as it is not elaborately constructed, as seen by the cuts shown in our spring, 1918, list. Meanwhile, for home use you can get along without a huller by using an old coffee mill or small hand grist-mill. In such cases the grain may be cleaned by shifting and pouring on a sheet in a current of air.

Prof. Hansen imported in 1898 a considerable quantity of seeds of this plant and energetically advocated the use of this product in South Dakota, at the same time pointing out its culinary value. He imported it for the first time in 1897–1898, making altogether 32 importations, but on account of lack of space we cannot give this list, which the reader may find in Bulletin 158 of the South Dakota Agricultural Experiment Station.

The most remarkable feature of Proso is its great drought-resistance, enabling it to be raised in places where even the best drought-resistant wheats cannot survive. I may quote briefly from the bulletin just mentioned:

Over a vast area of our western states the crop failures during the 4 years period (1910–1913) show with great force that we must increase the list of drought-resistant cereals. Wheat, oats, barley, rye—can we increase this list? As settlers go into the new, drier upland regions of our western states, from the Mexican boundary north to Canada they find that the staple small grains are less certain than in the more moist regions further east. In the drier interior region of Asia the grain that succeeds where all other grains fail, owing to drought, is Proso. In the dry interior desert north of the Sea of Aral in Turkestan and north into Siberia, Proso is a great food staple of the Kirghiz nomad—tent dwellers in the desert. It is a great advantage to have a grain like Proso that can be sown in the spring after it gets too late for other grains, a grain that will serve as a catch crop and yet ripen early enough to afford a satisfactory yield.

There are a number of varieties of Russian Proso, which, like the German and Italian millet are used for fodder.

The principal definitely named varieties at present known to us are Early Fortune, Manitoba, Black Voronezh, Red Voronezh, Red Russian, Tambov, Red Lump and Red Orenburg. Even some of these are very similar to each other and may be identical. All but the first two have been imported from Russia since 1897. Several so-called varieties making up our stock known previous to this period and imported largely from Germany, Austria-Hungary, China, and Japan may be distinct, but they have not yet been thoroughly studied.[8]

Hansen's White Siberian Millet. From Hansen's description (Northern Novelties for 1919) this is a large seeded, white grained millet of the Proso type. It is grown as a grain for stock by the Kirghiz Tartar nomads in the Semipalatinsk region, and it is also extensively eaten by these people. Hansen regards it as "the cornerstone of their agriculture in this 8-inch rainfall climate." In respect to its use in the United States, he says:

The farmers upon our driest uplands in the western states from the Mexican to our Canadian boundary should be able to raise this grain millet during our driest years. . . . . In 1914 we sowed 5 pounds per acre and harvested 22 bushels. The seed weighs up to 60 pounds per bushel. In this vicinity it has been grown on blue grass pasture broken up and seeded at the rate of one-half bushel

[8] Cyclopedia of American Agriculture, (edited by L. H. Bailey). Vol. 2, p. 471, 1907.

seed per acre as late as the 13th of July. Under these conditions a Brookings county farmer in 1914 raised a crop of nearly 43 bushels per acre.

Also, the Russian invention—the Proso huller—has now appeared in America, with great delay, to be sure (as was related above). This invention was also a gift from Russia, and the writer has had an opportunity of witnessing this machine at work in the City of Kief, N. D.—a Russian city in America.

Besides the white Russian millet mentioned above, the following varieties are also known: Early Fortune 72, White Ural 73, White Ural 320, Red Orenburg 75, Red Orenburg 321, Red Orenburg 333, Black Voronezh 76, Black Voronezh 319, Black Voronezh 331, Tambov 77, Tambov 366, S.P.I. No. 1062574, Red Voronezh 165, Red Lump 318, Red Russian 334.

The most serious experiments with Proso are being conducted at the Agricultural Experiment Station, Highmore, South Dakota. Data taken from Bulletin 101 (1907), Agricultural Experiment Station, Brookings, South Dakota (p. 148) gives the yield of Proso: the yield of several varieties for 1906 are included in Bulletin 101 of the South Dakota Agricultural Experiment Station, from which it will be seen that averaging 2 series of tests, the yields were from 31.8 to 43.3 bushels per acre, with straw yielding from 2180 to 2910 pounds.

7. Russian Flax

Flax is not a native of the United States. Of the flax grown in South Dakota we read in Bull. 169 (1916) of the state Agricultural Experiment Station the following:

> Although there are several types of flax grown in different parts of the world which differ materially in height of plant, color and size of flower and seed, all that is grown commercially in South Dakota comes from European sources. This type has small dark brown seeds, blue flowers at stems which are usually 18 to 24 inches in height.

Various types of flax to be found at experiment stations and on farms in the United States came from Europe, Asia, Africa, and South America, and are called in most cases by names derived from the places from which they are exported. The two main commercial varieties of flax are: (a) seed flax, a low plant that ripens early, and (b) fiber flax, high and ripening slowly. Although at experiment stations we find a great variety of Smyrna, Cretian, Egyptian, Indian, Abyssinian, and Argentine flax, on the other hand, on farmers' fields the predominating variety is the local, common flax most of which is obtained from Russia, where the greater part of seeding material is to be found in northern Caucasus and southern Russia. The question as to who first introduced flax into the Great Plains remains unanswered, although it is definitely known that Russian immigrants in their homesteads in North Dakota near the present cities of Kief, Max, Dogden, Ruso, Drake, and Keldee were the first to plant flax with success, and flax was, in fact, the principal agricultural product of Russian immigrants.

Conversations which the writer had with agricultural experts at experiment stations confirmed statements made by firms dealing in flax, that all flax seed is obtained from Russia. One thing that surprised the writer most, however, was

the statement made by Prof. Bolley that so-called Belgian and Dutch flax was also imported under the same name through Riga, that is, from Russia.

Russia, as is known, has two flax-growing regions: (a) a southern one in northern Caucasus and southern Russia—where flax is grown for seed only, and (b) a northern one, embracing the provinces of Pskov, Novgorod, Vologda, and the adjoining districts, where fiber flax is grown. While the southern region exports the seeds through Novorossiisk, Odessa, and other Black Sea ports, the northern region exports fiber, as well as seeds principally through Riga. In view of this Prof. Bolley's statement is, of course, quite plausible.

As regards the varieties of flax, the number has greatly increased in the United States, thanks to careful selection, so that varieties immune to fungus diseases now exist, the basic material being Russian varieties of seed flax. In South Dakota we find: Russian N. D. 155 (S. D. 155), Russian N. D. 155 (S. D. 691), Select Russian N. D. 608 (S. D. 154), Select Russian N. D. 1215 (S. D. 686). All these are distinctly Russian varieties, as is also their derivative N. D. Resistant 52 (S. D. 29).

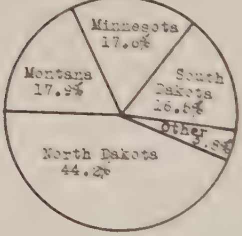

FIG. 9. Distribution of flax acreage in various states.

It is evident from the above list that most varieties of flax in South Dakota are imported from North Dakota, where the predominating varieties are Russian. Only one flax variety is imported from Budapest, the so-called N. D. Resistant 114 (imported into South Dakota under the name S. D. 688). This last-named variety is cultivated mostly on old lands (a small area in the eastern part of the state), whereas the Russian varieties of flax, N. D. Resistant 52 and N. D. 155, quotation from Bulletin 169 (1916) of the South Dakota Agricultural Experiment Station, is indicative of the results:

> Satisfactory yields of flax were secured on dry land at Newall in the year 1912 and 1915. Select Russian N. D. No. 1215 (S. D. 686) has given the highest average yield during the 4-year period. This variety was also originated at the North Dakota Experiment Station. The yields of Primost, Minn. No. 25 (S. D. 25) have been somewhat less than those of Russian varieties.

In the State of Wisconsin the writer observed excellent fiber flax of enormous height, and believing that Prof. Bolley is correct in his statement regarding the nativity of Belgian seeds, America may once again thank Russia. In Montana a considerable area is under flax, consisting almost entirely of Russian varieties named above. At the Montana Experiment Station, tests are continually being made with the different exotic varieties of flax, and new forms are originated by means of hybridization. Minnesota cultivates about as much flax as Montana, the number of varieties being about the same as in North Dakota. The Agricultural Experiment Station in Judith Valley, Moccasin, in the same state, is conducting experiments with flax on a large scale.

A comparison of varietal yields is given in Table 2.[9]

[9] From Bulletin 898, U. S. Department of Agriculture, by N. C. Nicholson

TABLE 2. ANNUAL AND AVERAGE YIELDS OF 19 VARIETIES OF FLAX GROWN IN PLATS AT THE JUDITH BASIN SUBSTATION, MOCCASIN, MONT., IN PERIODS OF VARYING LENGTH, 1911 TO 1915, INCLUSIVE

GROUP AND VARIETY	C. I. No.	Yield per acre (bushels).						
							Average.	
		1911	1912	1913	1914	1915	1911 to 1915	1914 to 1915
European seed:								
Russian (N. Dak. No. 155)	19	19.3	13.3	16.6	13.0	22.8	17.0	17.9
Russian (N. Dak. No. 155)	17	14.6	11.7	15.6	12.5	18.9	14.7	15.6
Select Russian (N. Dak. No. 608)	1	17.1	10.0	14.0	12.0	19.3	14.5	15.8
Select Russian (N. Dak. No. 609)	45	.....	.....	.....	13.6	19.0	....	16.3
Select Russian (N. Dak. No. 1215)	3	15.7	13.0	18.0	12.8	19.5	15.8	16.2
Montana Common	6	15.4	10.0	9.0	13.2	20.0	13.5	16.6
Select Riga (N. Dak. No. 1214)	2	18.3	9.0	19.6	13.0	19.1	15.8	16.1
Stepan (N. Dak. No. 1340)	5	15.0	11.3	16.8	12.1	19.9	15.0	16.0
North Dakota Resistant No. 52	8	16.0	6.3	14.0	11.8	19.5	13.7	15.7
North Dakota No. 1221	16	15.7	12.7	16.4	13.2	19.9	15.6	16.5
Fargo Common (N. Dak. No. 1133)	18	14.6	10.1	19.2	13.2	19.5	15.4	16.4
Smyrna seed:								
Smyrna	30	.....	.....	.....	14.8	19.2	....	17.0
Turkish	7	6.9	4.8	12.0	9.0	15.1	9.5	12.0
European short fiber:								
Kazan (N. Dak. No. 1329)	4	9.2	10.0	17.5	13.2	18.9	13.7	16.0
Idaho Common	15	.....	.....	.....	13.0	19.4	....	16.2
North Dakota Resistant No. 114	13	.....	.....	.....	12.3	16.8	....	14.6
Primost (Minn. No. 25)	12	11.0	10.3	17.5	12.8	19.1	14.1	16.0
European textile fiber:								
Blue Blossom	22	9.6	8.3	15.3	12.1	17.5	10.5	14.6
Pskof	32	.....	.....	.....	10.0	8.0	....	9.0

Discussing Table 2 (originally Table XXIII) Nicholson writes (p. 36):

Leading Varieties. Table XXIII shows that in both the 5-year and 2-year averages the varieties of the European seed-flax group yield more than those of the other groups. The 5 highest yielding strains for the 5-year period, C. I. Nos. 19, 3, 2, 16, and 18, all belong to the seed-flax group. The Russian, C. I. No. 19 (N. Dak. No. 155), is the highest yielder and the Select Russian, C. I. No. 3 (N. Dak. No. 1213), a selection from North Dakota No. 155, is second. C. I. Nos. 17 and 19 are both Russian (N. Dak. No. 155), but were received at Moccasin through different sources. C. I. No. 19 has given better results than C. I. No. 17. North Dakota No. 155 is a bulk lot of seed of Russian flax obtained by the North Dakota Agricultural Experiment Station in 1898. It has been grown and distributed since that time. C. I. Nos. 1, 3, and 45 are selections from this variety developed in the nursery at the North Dakota station.

Figure 16 in the same publication referred to above graphically illustrates the superiority of the Russian varieties.

The production of flaxseed in the United States, an average of 5 years, 1911 to 1915 inclusive, is 18,940,000 bushels.

8. Russian Sunflower

The sunflower is definitely an American plant, growing as a staple in Peru, and as a weed in North America. It was used as food by the Indians before white men came to this country. The sunflower was brought to Europe by the Spaniards, but development as a cultivated plant was on Russian soil.

The rituals of the religion predominating in Russia obliged adherents of the Greeko-Catholic Church to eat vegetable foods during prolonged periods. Even the fats forming a part of the food during these Lenten periods must be of vegetable origin. This protracted "vegetarian" régime of the Russian people necessitated a substitution for the animal fats, and such substitutes were found in the oil extracted from seeds of various plants, such as mustard (wild and cultivated), hemp, and sunflower. It is now impossible to say by whom and when sunflower was introduced into Russia. Its culture was carried on intensively and extended over the entire south and east of Russia, including northern Caucasus. By means of seminatural selection the best varieties were originated and specialized.

There are 2 principal lines of development of this plant: In one case the aim was to obtain the largest extract of oil, irrespective of the size of the seed—and as a rseult small-sized oil-seeds were obtained from a low plant. In another case the selector aimed at obtaining the largest seed, to be used as a toothsome article, and then the edible "shelled-in-the-mouth" variety was produced. This became a saleable product, was roasted like the American peanut, and was cracked and eaten by all classes of the population of Russia. The latter variety is of considerable height and reaches its most resplendent bloom in the Kuban territory, in northern Caucasus and in Bessarabia. There is also a third variety of sunflower with colored seeds, medium size.

It is definitely known that the Russian sunflower was reintroduced into America by Russian immigrants and this plant, together with Russian watermelons, flowers, proso, and a number of fowl and birds, makes the Russian farm in this country so different from the neighboring non-Russian farms.

Up to practically the present time no particular attention was shown the Russian sunflower, and only quite recently a number of experiments made with a view of investigating its silage properties as a substitute for corn (maize) unexpectedly produced quite favorable results. Corn is a success in every respect in the Corn Belt and even a little to the north of it, but is not so successful in the so-called Transition Zone, which corresponds to central Russia. A number of varieties of corn are gradually finding their way into this zone, thanks to the energetic efforts of American selectors. However, the search for maximum tonnage, on account of its use in silos, and the great height of the Russian sunflower has brought about the idea of making use of it.

In America we find 2 varieties of Russian sunflower—"Mammoth Russian" and "Russian Giant." The first experiments with these, conducted at various experiment stations produced unsatisfactory results, and for forage, sunflower was adjudged unfit. Experiments were continued, however, and finally the sunflower proved its fitness as silage. A study of the situation, published in 1921, is illuminating.

"A study of the publications of the experiment stations of the United States reveals the fact that very little work has been done with sunflowers. It also points out the further fact that practically all of the investigations reported were planned to find out the value of the seed or of the head. Up to the present, very little work has been done to determine the value of the entire plant.

"The 1896 report of the Maine Experiment Station gives the results of work by Bartlett, in which a green weight of sunflower heads of 13.5 tons and of the entire plant of 24.4 tons per acre was harvested. This author states that sunflower silage was readily eaten when fed to live stock in mixture with corn silage.

"The Vermont Experiment Station reports a green weight of 5.7 tons of sunflower heads per acre in 1893. No records of the yield of the whole plant are given and the investigator states, 'The stalks are shown by analysis to be too woody for use'."

Experiments by the Nebraska, New York, Nevada, and Canadian Experiment Stations were also summarized. In Montana, as well as in Wisconsin and in North Dakota, tests were made in raising the sunflower for silage, by testing the effect of close planting upon the tonnage of green forage. Experiments with sunflowers have been carried on by the Montana Experiment Station for 4 years, 1915 to 1918 inclusive.[10] The tests in 1915 and 1916 were preliminary, in order to find the yield of the crop, which was 36.8 tons in 1915 and 31.07 tons in 1916. In many cases, however, the yield was as high as 44.1 tons, as will be seen from the following table taken from Bull. No. 131, previously quoted.

TABLE 3. AVERAGE RESULTS OF PLANTING IN ROWS AT DIFFERENT DISTANCES, 1917 AND 1918

Distance between rows (inches)	Seed per acre (pounds)	Average height (inches)	Green forage per acre (tons)
8	30	101	39.8
20	12	96	32.5
24	10	93	26.7
30	8	112	33.6
36	5	112	44.1
42	4	102	23.0

Grown for silage in Washington, sunflowers produced approximately twice the yield of corn. These data represent an average of 2 years on experimental fields,[11] as indicated in Table 4.

TABLE 4. ACRE YIELD OF SUNFLOWER AND CORN SILAGE

Crop	Acre yield in tons		
	1919	1920	Average
Sunflowers	12.00	11.19	11.59
Corn	6.68	5.27	5.97

There is every reason to anticipate that the sunflower will be very extensively grown in the Transition Zone and that without any doubt Russian varieties will be used.

There has been a rapid growth in the cultivation of Russian varieties of sunflower for silage in sections of this country and of Canada. For the production of seeds alone the sunflower is grown only in small districts, largely in California and Missouri.

[10] Atkinson, Alfred. Montana Agr. Exp. Sta. Bull. 131, 1921.
[11] Washington Agr. Exp. Sta. Bull. 162, 1921.

9. Common Russian Alfalfas

Alfalfa is derived from Europe and Asia. In Asia its cultivation dates back to ancient times. Alfalfa was brought to America by Spaniards apparently and was first introduced into Peru and South America, later on appearing in California, where originally all alfalfa was of South American derivation, and where it is still called "Peruvian Alfalfa," representing quite a satisfactory variety for the southern part of California and Arizona. The eastern states obtained alfalfa from France, and according to some authorities, alfalfa was imported for use in these states by President George Washington.

When the pioneers began to move to the Far West, with its arid and colder climate, both known varieties of alfalfa proved unsatisfactory, and the moderately arid parts of the Great Plains received as a gift the so-called "Grimm Alfalfa," the origin of which has not yet been completely ascertained. There is, however, some information to the effect that this variety was exported by Wendelin Grimm in 1857 from Posen (now a part of Poland). This information was supplied to the writer by Bolley. Other information tends to show that Grimm alfalfa was obtained from Switzerland.

It is evident that the name "Grimm Alfalfa" has become a common one in the United States, and is frequently used to denote alfalfa mixtures, sometimes with pale blue flowers, growing fairly high. Some of this Grimm alfalfa is undoubtedly not of German or even Swiss origin, as it is entirely too drought-resistant, and is a result of breeding in the United States.

At the Experiment Station at Mandan, North Dakota, the author saw excellent specimens of the so-called Grimm alfalfa, which differed from Turkestan alfalfa by the larger number of seeds, paler shade of flowers, and height.

However, it may be definitely stated that the most extensive growing of alfalfa in this country did not begin until the expedition of the eminent Prof. N. E. Hansen, whose name is closely associated with alfalfa in the United States. Moreover, this plant was a large factor in the task of colonizing the arid regions of the Middle and Far West.

In his investigations, looking toward the importation of useful plants, Hansen was guided by considerations of similarity of climate between the Great Plains and certain parts of Russia. One of his first guiding principles was that obtained under the influence of De Candolle. I quote excerpts from a statement of Hansen regarding hardiness made in 1912.

> Hardiness against severe cold is a question of heredity. No perfectly hardy alfalfa has ever been developed in the mild climate of southern Europe or southern Asia.
>
> The "acclimatization" of a tender alfalfa is a myth. Acclimatization is a sieve that sifts out less hardy strains, but it does not put into the sieve anything not there in the first place.
>
> We can get our perfectly hardy alfalfa only from climates similar to our own in extremes of winter cold. In other words, *Acclimation* (Nature's work) is possible; *Acclimatization* (Man's work) is impossible.

The search for climatic and soil conditions similar to those in the American Great Plains brought Prof. Hansen to Russia. His favorite district there is Semi-

palatinsk, where the July isotherm rises far to the north, and the January isotherm comes down considerably to the south.

To what extent Prof. Hansen was influenced by the Russian Mennonites and their Russian crops in South Dakota in the counties Hanson, Davison, Huron (near Lake Baron), Edmunds, and McPherson, it is difficult for the author to say.

The following varieties of Russian alfalfa introduced by Hansen and now cultivated in the United States belong to 2 botanical species: (1) *Medicago sativa*, to which belong the French, Grimm, Turkestan, Peruvian and all other alfalfas with blue flowers; (2) *Medicago falcata*, with yellow flowers, which Prof. Hansen was the first to bring from Russia.

To these should be added the hybrid between the above 2 forms, the *Medicago media*, which was obtained as a result of cross pollination. Another alfalfa at present cultivated, in small quantities as yet, is *Medicago ruthenica*, also brought from Russia by Hansen. The following are some of the Hansen alfalfas. (Cf. Some new alfalfas, 1912):

Select Turkestan Alfalfa. This is *Medicago sativa Turkestanica*, No. 191 of my 1906 trip (S. P. I. 20711), originally developed from seed of a single plant found at Tashkend, the capital of Russian Turkestan. This plant is remarkable for its erect and vigorous growth. At Moscow it was found very hardy and productive, a beautiful plant, where the French lucern, by which is meant the ordinary cultivated alfalfa of southern Europe, winter-killed. This variety will be appreciated wherever the Turkestan alfalfa is found fully hardy.

Cossack Alfalfa. This is my No. 194 of my trip (S. P. I. No. 20714). A sand Lucern (*Medicago media*), a hybrid alfalfa from the Voronesh (or Voronezh) province of the Don river region of southeastern Russia. This spontaneous or natural hybrid of *M. falcata* and *M. sativa* will sometimes have blue flowers on one branch, yellow on another, sometimes both colors on the same branch; a heavy seeder the past 3 years.

Cherno Alfalfa. This is my No. 196 of the 1906 trip (S. P. I. 20716). A Sand Lucern or hybrid alfalfa (*Medicago media*) descended originally from a single plant found wild on the steppes of the Voronesh province, southeastern Russia, land of the Don Cossacks. The flowers are called black-green, but are really a very dark purple changing to a rich green with dark purple veins; plant of strong very upright growth, a heavy seeder here the past three years.

White Flowered Alfalfa. The variety here offered distinguishes itself by strong upright growth and productiveness both as to forage and seed. It is a beginning only. In 1916 we found that the seed came fully 70 per cent true to the white color, but the work may easily be completed. Owing to lack of room the seed is now offered to experimenters elsewhere. The seed may be sown in rows and the plants transplanted after one year's growth as described in my bulletin 167. The plants that do not come true as to white color of flowers should be removed as soon as they show the off color. This variety originated as a seedling of the yellow-flowered alfalfa, *Medicago falcata*, from Omsk, Siberia, grown closely adjacent to the Cossack.

Semipalatinsk Alfalfa. Plants grown from seed gathered from wild plants in the dry steppes of the Semipalatinsk region, Akmolinsk province, southwestern Siberia. Plants are mostly of the tall growing type much the same as the lot gathered in 1908 on the Irtysh river about ten miles north of Semipalatinsk, (S. P. I. 24455), from plants of erect habit, with stems some of which were five feet eight inches long. Flowers bright yellow. This is *Medicago falcata* from a region with very cold winters and dry hot summers.

Samara Alfalfa. This is my No. 201 of the 1906 trip (S. P. I. 20721); of tall erect growth with beautiful yellow flowers *M. falcata*. From the dry steppes of Samara province in the Volga river region of eastern Russia. This may range further south than the Omsk and Obb Siberia strains, but should be found drought-resistant and sufficiently hardy for South Dakota.

Omsk 1908 Siberia Alfalfa. Hansen's *Omsk Siberia* Alfalfa (1908 seed). This was grown from seed gathered from wild plants near Omsk, Akmolinsk province, western Siberia (S. P. I. 24453) in my 1908 trip to Siberia, hence is really the same as my No. 199 of my 1906 trip (S. P. I. 20719) *Medicago falcata* gathered in the same place. The plants hold their own perfectly with other native plants in the compact prairie or steppe sod. Omsk is in latitude fifty-five degrees. A plant of vigorous habit with bright yellow flowers. The plant varies somewhat in erectness of habit so that there is room for improvement by selection.

Obb Siberia Alfalfa. This is *Medicago falcata* gathered in my 1908 trip (S. P. I. 24452) on the open steppes near Obb on the Obb or Obi river of the Tomsk province, central Siberia. In hardiness and general characteristics it is much like the Omsk Siberia strain.

Orenburg Alfalfa. This is my No. 261 of my third tour to Siberia, 1908. This is *Medicago falcata*, grown from seed gathered for me from plants growing wild in the dry steppe region at Orenburg, Orenburg province, on the extreme eastern border of European Russia. Summer heat of 98 degrees above, and winter cold of 33 degrees below zero Fahrenheit are not uncommon. The annual rainfall at Orenburg is a little less than 16 inches; in this region the yield of hay from this wild yellow-flowered alfalfa is reported at 300 Russian "pood" per dessiatine, which equals two tons per acre, and the yield of seed 26 pood per dessiatine, or 348 pounds per acre.

Distribution. Russian varieties of alfalfa are gradually obtaining wide dis tribution. Prof. Hansen thinks that *M. falcata* and its varieties can be cultivated as far north as the Yukon, Canada, and Alaska. A most valuable feature of the Russian varieties of alfalfa is the drought-resistance; and of *M. falcata* and its varieties, the frost-resistance. The most peculiar feature of the Semipalatinsk alfalfa is the great height of each individual plant, which prevents close sowing and considerably minimizes the seed expenditures.

It is quite true that the improved variety called "Grimm" is of perfect quality and competes with the commercial mixture of Russian common alfalfas called usually "Turkestan alfalfa." But the history of the colonization and the conquest of the Northwest is connected with the extreme drought-resistant Turkestan alfalfa.

10. Russian Forage Grasses

The wider known Russian grasses imported into America by representatives of the U. S. Department of Agriculture, as well as by private individuals are: Bromegrass (*Bromus inermis*), imported by Prof. Hansen, Wheat Grass (*Triticum cristatum*), some varieties of timothy and *Lasiagrostis splendens*, of which the first is already cultivated and the second is being tested on experimental fields in the Northwest. At the Cornell University Experiment Station, where selection and hybridization tests of timothy are conducted on a large scale, many Russian varieties are under investigation, seeds having been contributed by Russian agriculturists in the United States

VARIETAL, CULTURAL, AND SEASONAL EFFECTS UPON THE WATER REQUIREMENT OF CROPS[1]

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It has been the purpose of these investigations to determine the general principles pertaining to the use of water by crops, especially maize, in relation to plant type, seed source, soil fertility, soil moisture, and seasonal climatic differences. It was not primarily the intention to establish these relationships for specific varieties or environmental conditions, but rather to develop information which would be generally applicable.

The ratio of the weight of water transpired to the moisture-free plant substance produced is known as the "water-requirement ratio" and has come to be regarded as the most important of the transpiration relationships. It is often spoken of as an index of the degree of economy or extravagance in the use of water by the crop. In the case of species and varieties under comparison the water-requirement ratio is sometimes referred to as indicating relative drought resistance. In the case of cultural treatments or fertilizer applications a reduction in this ratio has been interpreted as facilitating crop production under dry-land conditions through lowering the total amount of water needed.

In connection with these investigations we may seek the reliability of these interpretations.

Technique of these Investigations

All tests were made under control conditions, during the normal crop season, and with reasonably normal field exposure. It was necessary to grow the plants in potometers in order to permit measurement of the amount of water used. Except where otherwise specified in certain "Method studies," the galvanized iron pots were 16 inches in diameter and 36 inches deep, and held approximately 250 pounds of moisture-free soil. These were filled with packed soil to within 3½ inches of the top. The soil was covered with a 3½ inch layer of fine gravel to check surface evaporation. A galvanized iron lid with central opening for plants was placed over each pot of corn, sorghum, and sunflowers. As soon as the plants attained sufficient size, this central opening of the lid was sealed with oilcloth and plastic modeling clay which served to exclude precipitation. In the case of small grain and alfalfa grown in comparison with the corn, rain was excluded by

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 18, 1926.

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means of a temporary canvas roof, which could be rapidly lowered over a simple frame-work whenever rain threatened.

An effort was made to grow the various crops at planting rates fairly comparable with field conditions. Thus 1 corn, 1 sunflower, 2 sorghum, 100 wheat, and 100 oats plants were grown per pot. Except in the soil moisture studies, a constant and favorable supply of soil moisture was maintained by adding weighed quantities of water to a covered, glazed stone jar connected by rubber tubing with the base of each potometer. The water used by the crop was determined from the difference in initial and final weight of the potometer and from the net water supplied during growth. In case of the soil moisture studies the amount of water transpired was replaced daily through a perforated coil buried within the soil mass. Use of the coil avoided localization of small water applications.

The potometers were placed in excavations in the field, with their tops level with the adjacent land. Normal wind protection was afforded by planting the surrounding field to crops similar to those tested in the potometers. Grown under these conditions the various crops yielded in a very satisfactory manner and in accordance with the favorableness of conditions. Plant development and grain yield were fully equal to farm production.

Inconstancy of Water Requirement Ratios

While various kinds of crops may differ inherently in their water economy, they should not be thought of as having fixed water-requirement ratios per pound of dry matter. Such ratios have differed decidedly in these experiments for any one crop. For example, as extreme seasonal effects upon corn, the ratio per pound of dry matter was 192 in 1915 and 444 in 1913. Variation in the exposure of the potometers has resulted in a 6-year average variation of 270 for corn plants surrounded by a corn field, to 340 for corn plants fully exposed in the open. Variations in soil-moisture content have caused the water-requirement ratios of corn to vary from 289 to 341 as an average for 3 years. The water-requirement ratio of corn was 286 when grown in the surface foot of soil and 518 for the third foot of soil, as an average for a 5-year period.

VARIETAL EFFECTS IN CORN

Plant type. Four commercial varieties of dent corn which differ in vegetative size and earliness were tested during the 7-year period, 1914 to 1920 (Table 1).

TABLE 1. RELATION OF PLANT SIZE AND EARLINESS TO THE WATER REQUIREMENT OF CORN AVERAGE FOR 7 YEARS, 1914–1920

Description	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq.in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Hogue Yellow Dent	56	101	1176	196	476	126.2	657	269
University No. 3	27	95	1066	159	398	109.0	710	278
Schmalle's Pride of the North	27	89	894	168	394	104.1	643	269
Disco Pride of the North	25	66	514	109	236	63.4	602	279

[1] Average of ratios.

The varieties included (1) Hogue Yellow Dent, which is a standard full-season crop adapted to southeastern Nebraska; (2) University No. 3, which originated as a medium-early ear-to-row strain of northeastern Nebraska Reid Yellow Dent; (3) Schmalle's Pride of the North, which is a rather early eastern Nebraska variety suitable for late planting, and (4) Disco Pride, which is a very small early type grown in South Dakota. Although the smallest variety differed only 3 per cent from the largest variety in the water-requirement ratio, it used only 50 per cent as much water per plant. While the slight variations in water requirement indicate about equal efficiency for the different types, the data clearly suggest that low total water consumption per plant is associated with small plant size.

Regional types of corn,—Florida to Washington. It is a well-known fact that varieties of dent corn become smaller and earlier as one progresses diagonally across the United States from Florida to the State of Washington. This change in plant type is associated with a corresponding transition in the length of growing season and amount of available precipitation.

Representative varieties were tested in 1924 from the states of Florida, Alabama, Missouri, Nebraska, Wyoming, Idaho, and Washington (Table 2). Three

TABLE 2. COMPARATIVE WATER REQUIREMENTS OF REGIONAL TYPES OF CORN, 1924

Source of Seed	Plant height	Leaf area per plant	Date in tassel	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain	Gram dry matter
	Ins.	Sq.in.		Gms.	Gms.	Kgms.	Gms.	Gms.
Florida	117	1564	8/10	174	542	159.5	917	294
Alabama	119	1650	8/13	167	493	154.3	924	313
Missouri	112	1296	7/31	226	482	142.2	629	295
Nebr. (Lincoln)	106	1127	7/26	242	479	127.6	527	266
Nebr. (North Platte)	83	853	7/23	176	365	94.8	539	260
Nebr. (Kimball)	58	421	7/13	119	242	60.0	504	248
Wyoming	63	412	7/14	116	233	59.3	511	255
Idaho	63	333	7/11	106	212	54.5	514	257
Washington	58	317	7/12	104	206	52.2	502	253

varieties typical of eastern, central, and western Nebraska were included. Rather normal growth was obtained for all sorts. Compared with corn from Idaho and Washington, the plants from Florida and Alabama were nearly twice as tall, came into tassel a month later, had approximately 5 times the leaf area per plant, transpired 3 times as much total water per plant, and their water-requirement ratio per unit of dry matter was about 20 per cent greater. The total plant weight was also approximately $2\frac{1}{2}$ times as large.

From the standpoint of suitability for dry-land conditions, the small northwestern varieties may be regarded as advantageous, due more to the decidedly smaller amount of water used per plant, rather than to a lower water requirement in dry-matter production. It seems probable in a test of this kind that the very large late-maturing varieties indicate a relatively somewhat too large water-requirement ratio, due to the restricted quantity of soil per pot becoming

more of a limiting factor in production. The varieties from the 3 most southern states may also have been at some disadvantage because of slight immaturity. There was little significant difference in the water-requirement ratios of varieties acclimated to regions between southeastern Nebraska and the state of Washington, although there was a decided difference in the total water consumption per plant.

Bearing these qualifications in mind, there is little evidence of material inherent differences in the amount of water transpired per pound of dry matter in relation to the conditions under which the corn has become acclimated.

Humid, sub-humid, and semi-arid types of corn. Two regionally acclimated varieties from each of 3 localities, New York, eastern Nebraska, and western Nebraska, were tested during the 7-year period, 1914–1920 (Table 3). These 3 sets may be thought of as acclimated to humid, sub-humid, and semi-arid conditions, respectively, having long been grown in these regions. No significant difference was found in the water-requirement ratios.

TABLE 3. EFFECT OF ACCLIMATIZATION UPON THE WATER REQUIREMENT OF CORN AVERAGE FOR 7 YEARS, 1914–1920

Description	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
New York (humid)								
Wood's White Dent	23	93	1104	145	416	106.8	775	260
Rocky Mountain Dent	27	91	871	141	363	96.4	689	266
Average	..	92	988	143	390	101.6	732	263
Eastern Nebraska (sub-humid)								
Hogue Yellow Dent	56	101	1176	196	476	126.2	657	269
University No. 3	27	95	1066	159	398	109.0	710	278
Average	..	98	1121	178	437	117.6	684	274
Western Nebraska (semi-arid)								
Calico	26	84	948	166	392	103.5	647	269
Marteens' Dent	28	72	673	115	280	72.2	654	262
Average	......	78	811	141	336	87.9	651	266

[1] Average of ratios.

Varieties of corn described as drought resistant. During a 7-year period, 1914 to 1920, 2 varieties of corn, Chinese and Esperanza, which have been described by G. N. Collins as being especially drought resistant, were compared with the Sherrod dent variety from central Kansas, and with a hybrid selection made by H. F. Roberts which involved all 3 of these varieties (Table 4). There was no significant difference in the water-requirement ratios of the Chinese, Sherrod dent and Roberts hybrid. The ratio of Esperanza corn was relatively high,

doubtless due to poor production. The variety has never produced well under Nebraska conditions, due in part to late maturity.

TABLE 4. COMPARATIVE WATER REQUIREMENTS OF SPECIAL DROUGHT-RESISTANT VARIETIES OF CORN—AVERAGE FOR 7 YEARS, 1914–1920

Description	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Chinese	25	73	1098	88	341	91.6	1684	274
Esperanza	23	91	1021	33	309	96.1	4864	317
Sherrod (Kansas dent)	26	82	939	168	397	103.6	617	266
Roberts Chinese×Esperanza× Sherrod Hybrid	28	85	1072	130	387	104.3	833	268

[1] Average of ratios.

The water-requirement ratios of this test fail to reflect any qualities which may be interpreted as special drought resistance.

COMPARISON OF CORN WITH OTHER CROPS

Corn, wheat, and oats. Because of the great acreage of wheat and oats grown in this country under conditions of limited rainfall, a comparison of the water requirement of these crops with that of corn was thought to be of interest.

During two years, 1915 and 1916, these crops were compared (Table 5) under similar soil and moisture conditions and with exposures normal to the respective crops. The small grain pots were placed in narrow trenches on a level with the oat and wheat fields in which they were grown, at a distance of 10 rods from the corn. Standard local varieties were used, and satisfactory growth and yields procured. The respective water requirement ratios for Hogue corn, Turkey Red winter wheat, and Kherson oats were 230, 321, and 426.

TABLE 5. COMPARATIVE WATER REQUIREMENTS OF CORN, WINTER WHEAT, AND OATS SURROUNDED IN THE FIELD BY THEIR RESPECTIVE CROPS TWO-YEAR AVERAGE, 1915—1916

Crop	Total no. of pots	Plants grown per pot	Dry matter		Ratio of grain to stover or straw	Transpiration per		
			Grain	Total		Pot	Gram grain[1]	Gram dry matter[1]
			Gms.	Gms.		Kgms.	Gms.	Gms.
Hogue Corn	16	1	203	527	.63	120.2	599	230
Turkey Red Wheat	12	100	54	197	.36	61.0	1236	321
Kherson oats	12	100	63	170	.58	72.0	1157	426

[1] Average of ratios.

It is generally considered that these small grain crops dry out the soil more than does corn, and their large water-requirements may perhaps help to account for this.

Alfalfa compared with other crops. Alfalfa, milo, and wild sunflowers were tested in 1916 in comparison with the other crops. The data are presented in

Table 6. The alfalfa potometers stood in a narrow trench in an adjacent alfalfa field, while the milo and sunflowers were in the same pit as the corn. Although alfalfa is grown most extensively in territory where moisture shortage is common, it has by far the greatest water-requirement ratio of any of the crops tested. This high water-requirement, together with high acre-production, will account for the excessive depletion of soil-moisture to a depth of 15 feet or more under typical eastern Nebraska farm conditions. This is made possible by the deep penetration of alfalfa roots.

TABLE 6. COMPARATIVE WATER REQUIREMENT OF ALFALFA[1] AND OTHER CROPS WHEN GROWN UNDER NORMAL EXPOSURE IN FIELDS OF THEIR OWN RESPECTIVE CROPS, 1916

Crop and Variety	Total no. of pots[2]	Dry matter per pot	Transpiration per	
			Pot	Gram dry matter
		Gms.	Kgms.	Gms.
Milo (dwarf)	4	194	51.3	264
Corn (Hogue)	8	513	137.0	267
Wheat (Turkey)	6	251	71.0	323
Oats (Kherson)	6	188	77.9	414
Sunflower (wild)[3]	4	620	350.3	565
Alfalfa (Common)[1]	6	201	172.4	858

[1] One-year-old alfalfa.
[2] Pots 16 inches in diameter by 36 inches deep.
[3] Helianthus annuus.

Corn and sorghum compared. Sorghum is generally credited with being more drought-resistant than corn and is regarded as more suitable to extensive semi-arid sections of Kansas, Oklahoma, and Texas, because of superior resistance to moisture-shortage conditions. A comparison of these 2 crops was therefore thought to be of special interest, as indicating whether the water-requirement ratio might serve as a measure or indicator of this recognized difference in dry-land adaptation. Accordingly, 3 standard varieties of sorghum, namely, Black Amber, Feterita, and Dwarf Milo, were compared with the standard local variety of Hogue Yellow Dent corn during the 7-year period 1914–1920 (Table 7). The water requirement per gram of dry matter averaged 304 for the 3 varieties of sorghum, compared with 269 for corn. Milo proved to be the most productive sorghum in these tests, and its water requirement was 273. Thus it appears that the water-requirement ratio obtained in this manner provides no clue as to an inherent difference in drought resistance of these crops.

The individual sorghum plants were smaller and averaged less than half as much water consumption per plant as the corn.

In order to determine whether a different transpiration relationship might exist under conditions of actual moisture shortage, Hogue corn and Milo were compared during 2 years under 4 degrees of soil-moisture content (Table 8).

Based upon the total amount of water required to saturate the soil against seepage, the variations in soil-moisture content maintained constantly in this test were 40, 50, and 70 per cent relative saturation. In a fourth set an artificial

TABLE 7. COMPARATIVE WATER REQUIREMENTS OF CORN AND SORGHUMS AVERAGE FOR 7 YEARS, 1914—1920

Crop	Total no. of pots	Dry matter		Transpiration per		
		Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Gms.	Gms.	Kgms.	Gms.	Gms.
Hogue Yellow Dent corn	56	196	476	126.2	657	269
Black Amber sorghum	28	47	143	44.6	1012	315
Feterita	28	52	185	59.1	1251	323
Milo	27	101	249	68.5	728	273
Average for sorghums				57.4	997	304

[1] Average of ratios.

drought was introduced shortly before the corn came into silk and before heading of the Milo. The water was supplied at the optimum rate of 70 per cent until this period when it was entirely withheld, till decided wilting occurred. Favorable moisture condition was then restored. The yields of grain and stover indicate distinctly that the 40 and 50 per cent saturations and the artificial drought constituted unfavorably dry conditions. The 40, 50, and 70 per cent saturation and the artificial drought resulted in water-requirement ratios of 193, 201, 216, and 236, in case of the corn, and 236, 249, 243, and 261 grams in the case of the milo.

TABLE 8. COMPARATIVE WATER REQUIREMENTS OF CORN AND MILO WHEN GROWN IN VARIOUS DEGREES OF SOIL MOISTURE AVERAGE FOR 2 YEARS, 1915—1916

Crop	Total no. of pots	Dry matter		Transpiration per		
		Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Gms.	Gms.	Kgms.	Gms.	Gms.
Forty per cent soil saturation						
Corn	7	133	315	59.3	452	193
Milo	8	70	188	43.4	621	236
Fifty per cent soil saturation						
Corn	8	170	395	77.7	460	201
Milo	8	79	226	56.6	785	249
Seventy per cent soil saturation						
Corn	7	212	489	105.4	495	216
Milo	8	114	312	76.9	679	243
Artificial drought at start of fruiting period						
Corn	8	58	270	64.1	1089	236
Milo	8	39	197	51.1	1847	261

[1] Average of ratios.

Based on the water requirement of corn at the 40, 50, 70, and "drought" percentages, the sorghum used respectively 122, 124, 114, and 111 per cent as

much water per unit of dry matter as the corn. In no case did sorghum have the lower water requirement. It appears that sorghum, with its recognized superior drought resistance, possesses this virtue by reason of other physiological qualities than low water-requirement ratio, which can not be detected in a test of this kind.

SOIL FERTILITY EFFECTS

At the time these investigations were begun, the theory had been advanced by a number of workers that crop production could be enhanced, in regions of too limited rainfall, through an increase in soil fertility by virtue of increasing the concentration of the soil solution and permitting the intake of the necessary amount of nutrient solutes with less water. Such a theory presupposes regulation on the part of the plant of the outgo of water, in accordance with the ease of acquiring the necessary amount of nutrients. It assumes that soil solutions are taken into the plant in the same density as these occur in the soil and that transpiration may be practically withheld in the presence of a very rich solution.

This theory is well known to be contrary to farm experience. Crop growers throughout the semi-arid region are cautious in the use of barnyard manure and legume-rotation systems which tend to enrich the soil, for fear of "burning up the crop," as they speak of it. Commercial fertilizers have the same effect. The crop "burning" appears to be due to stimulated vegetative development with consequent greater moisture demand by the plant and hastened moisture depletion.

It is believed that these tests confirm such farm experience and that a suitable balance should be sought between the fertility of the soil and the available moisture supply. When fertility is a limiting factor under prevailing moisture conditions, reasonable practices should be followed which will overcome the deficiency, thereby commonly lowering the water-requirement ratio. It appears that this can in no way be accounted for as due to transpiration regulation or control, because actually more water is transpired per plant. The lowering of the water-requirement ratio is merely an incidental, accompanying result of more thrifty and productive growth without a proportional increase in the amount of water consumed. Increasing the density of the soil solution does not lower the amount of transpiration of the plant until the amount becomes toxic, thereby dwarfing the plant and lowering the amount of water used in proportion.

Thus increased production through increased fertility is not brought about through lowered transpiration. The following tests are presented as evidence of these relations.

Surface vs. subsoil. During a 5-year period, 1915–1919, corn was grown in pots filled respectively with the surface foot, second foot, and third foot of soil, and with equal quantities of each foot replaced in natural order. Tests were made both with and without manure applications. The soil was typical Waukesha silt loam taken from a field of the Experiment Station farm, capable of producing 75 bushels per acre in a favorable season.

The differences in productivity of the soil taken at these different depths is best illustrated by the plant size and yields obtained, as shown in Table 9.

TABLE 9. COMPARATIVE WATER REQUIREMENTS OF CORN (PRIDE OF THE NORTH) WHEN GROWN ON SURFACE SOIL VS. SUBSOIL TAKEN FROM THE NEBRASKA EXPERIMENT STATION FARM—AVERAGE FOR 5 YEARS, 1915–1919

DEPTH OF SOIL USED	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq.in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Soil without manure								
First foot	19	85	794	129	316	88.2	755	286
Second foot	20	78	511	27	100	39.3	2580	410
Third foot	20	68	462	3	66	30.4	10133	518
Average	......	77	589	53	160	52.6	4489	405
Three feet	20	85	844	73	213	73.0	1427	374
Soil with manure								
First foot	19	88	880	151	349	101.4	686	294
Second foot	19	84	712	61	196	68.4	1799	359
Third foot	19	78	691	46	161	61.3	1852	400
Average	......	83	761	86	235	80.1	1446	351
Three feet	20	85	889	115	289	88.9	818	313

[1] Average of ratios.

Without manure, the third, second, and first foot of soil yielded 66, 100, and 316 grams dry matter per plant; produced 3, 27, and 129 grams of grain; transpired 30, 39, and 88 kilos water per plant, and 518, 410, and 286 grams per gram total dry matter, and 10133, 2580, and 755 grams per gram grain production. When manure was applied the plant size and weight and total water consumption were materially increased. For the third and second foot, respectively, this weight increase amounted to 144 per cent, 96 per cent, and 11 per cent. The increase in total water transpired resulting from the manure was progressively 102, 74, and 15 per cent for the third, second, and first foot.

The transpiration per gram dry matter was reduced 23, and 12 per cent respectively by applying manure to the third and second foot of soil and was increased 3 per cent by manure application to the first or surface foot.

On the basis of grain production, manure increased the yield of the third foot of soil 1400 per cent, the total water transpired 102 per cent, and lowered the transpiration per gram grain to 18 per cent of the unmanured soil.

When the soil was refilled in the pots in natural order to a depth of 3 feet, the increases from the manure application were 36 per cent in total dry matter, 58 per cent in grain yield, 22 per cent in the total amount of water transpired per plant, while the water-requirement ratios for grain and total dry matter were lowered 43 per cent and 16 per cent respectively.

Three degrees of soil fertility—with and without manure. During an 8-year period the medium-small variety of corn, Pride of the North, was grown in potometers filled respectively with infertile, intermediate, and fertile soil. The

transpiration determinations were made on both manured and unmanured soil (Table 10).

TABLE 10. RELATION OF SOIL FERTILITY TO THE WATER REQUIREMENT OF CORN (PRIDE OF THE NORTH)—AVERAGE FOR 8 YEARS, 1911–1918

Character of the Soil	Total no. of pots	Stalk height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq.in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Soil without manure								
Infertile	31	68	457	17	72	38.3	8263	531
Intermediate	32	77	554	26	108	51.3	2485	489
Fertile	32	87	715	74	213	77.4	1100	368
Soil with manure								
Infertile	32	82	658	52	180	68.9	1518	396
Intermediate	32	83	719	67	207	75.6	1173	369
Fertile	31	89	794	108	289	94.2	879	327

[1] Average of ratios.

The moisture-free weights of plants grown in the unmanured infertile, intermediate, and fertile soils were 72, 108, and 213 grams respectively; the transpiration per plant was 38, 51, and 77 kilograms. The corresponding water requirements per gram total dry matter were 531, 489, and 368 grams, and the water requirement per gram of grain was 8263, 2485, and 1100 grams, respectively. Applying manure to these 3 soils increased the total dry matter per plant 150 per cent, 90 per cent, and 36 per cent, respectively. The total water used per plant was correspondingly increased 80 per cent, 46 per cent, and 22 per cent, while the water-requirement ratio per unit dry matter was lowered 25 per cent, 28 per cent, and 11 per cent, respectively, for the infertile, intermediate, and fertile soil.

Effect of varying rates of manure application. As an additional test bearing upon the question of the relation of the strength of soil solution to the water-requirement of corn, manure was applied at varying rates to a series of potometers filled with productive surface soil from the Experiment Station farm (Table 11). The series represented (1) no manure, (2) normal amounts of manure, at the rate of 2.4 pounds (moisture-free) per plant, (3) 5 times the normal rate, and (4) 10 times the normal rate. The extreme effect was with the 5-fold manure application, which resulted in lowering the water-requirement ratio 7 per cent, accompanied by an increase in total transpiration of 6 per cent. Manure applied at 10 times the normal rate lowered the yield about 25 per cent below the 5-fold application but did not affect the water-requirement ratio appreciably.

Increasing the strength of soil solution through the application of manure up to the point of maximum production in no way served to lower the amount of water used per plant.

TABLE 11. EFFECT OF APPLYING MANURE AT VARIOUS RATES UPON THE WATER REQUIREMENT OF CORN (HOGUE)
AVERAGE FOR 7 YEARS, 1916–1922

RATE OF MANURE APPLICATION	Total no. of pots	Moisture free manure	Plant height	Leaf area	Dry matter		Transpiration per		
					Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Lbs.	Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
None	40	0	99	1068	185	418	116.2	634	280
Normal rate	52	2.4	98	1093	191	467	127.5	680	275
5 times normal rate	32	12.0	100	1127	197	478	122.9	642	260
10 times normal rate	32	24.0	88	1035	150	367	95.0	698	263

[1] Average of ratios.

In a corresponding 6-year test with a complete commercial fertilizer, dried blood, steamed bone meal, and KCl were applied in equal proportions of N, P, and K. The potometers were refilled annually with soil taken in natural order from the Experiment Station farm, to a depth of 3 feet. There was no tendency for increased fertility up to 10 times the normal rate to reduce the amount of water used per plant. Associated with a rather gradual increase in dry matter produced, there was a reduction in the water-requirement ratio from 363 for the unfertilized to 261 for the soil receiving a 10-fold application. The maximum grain and total production were also derived from this application.

TABLE 12. EFFECT OF APPLYING ARTIFICIAL FERTILIZER AT VARIOUS RATES UPON THE WATER REQUIREMENT OF CORN (HOGUE)
AVERAGE FOR 6 YEARS, 1917–1922

RATE OF FERTILIZER APPLICATION[2]	Fertilizer per pot	Total no. of. pots	Plant height	Leaf area	Dry matter		Transpiration per		
					Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
	Gms.		Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
None	0	28	89	828	109	275	88.0	1323	365
Normal rate fertilizer	35	25	92	826	129	326	100.6	1257	330
5 times normal fertilizer	175	21	93	892	149	365	105.3	858	301
10 times normal fertilizer	350	25	92	925	191	410	104.2	560	261
Normal rate sheep manure	1090	26	90	861	154	350	103.6	760	312

[1] Average of ratios.
[2] Fertilizer consisted of dried blood, steamed bone meal and KCl in amounts equivalent to equal portions of N, P, and K.

Taking all of the data bearing upon these fertilizer tests into consideration, it appears that the lowering of the water-requirement ratio is incidental and coincident to the more favorable production, and is merely a function of high yield (Table 12).

EFFECT OF VARIATIONS IN SOIL-MOISTURE CONTENT

The theory has frequently been presented that the ease with which water is diffused through the stomata of plants is adjusted by them in accordance with the abundance of soil moisture available to them. In pursuance of this relationship a number of tests have been conducted at the Nebraska Station to determine

the effects of variations in soil-moisture content upon the growth and the total water transpired per plant and per unit dry matter. The soil-moisture contents are expressed in percentage of the amount of water required for saturation, or that retained against seepage in this type of container and soil. The weight of water required to saturate averaged 40 per cent of the moisture-free weight of soil. Under these conditions 70 per cent relative saturation is regarded as optimum. The non-available moisture of this soil has been estimated at 12 per cent.

During a 5-year period Hogue Yellow Dent corn was grown at an optimum soil-moisture content which approximates 70 per cent saturation for this soil. In comparison, other potometers were cropped at 50 per cent relative saturation. The latter soil proved too dry for maximum yield (Table 13) as indicated by

TABLE 13. EFFECT OF LIMITATION OF SOIL MOISTURE UPON THE WATER REQUIREMENT OF CORN (HOGUE).
AVERAGE FOR 5 YEARS, 1910, 1913–1916

Condition of Soil	Soil saturation	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
					Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
	Pct.		Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Too dry	50	23	88	1061	167	385	94.4	601	254
Favorable	70	23	109	1200	223	509	139.5	628	276

[1] Average of ratios.

24 per cent reduction in total dry matter, 25 per cent reduction in grain yield, and 21 inches reduction in plant height. At the expense of one-fourth reduction in yield, these smaller plants grown on the drier soil used 8 per cent less water per pound of dry matter, 4 per cent less per gram of grain, and 33 per cent less total water.

During 3 of the 5 years reported in the foregoing test, an additional rate of 95 per cent relative soil saturation was included to represent a soil too wet for maximum production. That it proved to be so is indicated by a reduction of 19 per cent in total plant weight and 27 per cent in grain yield (Table 14). This

TABLE 14. RELATION OF SOIL MOISTURE TO THE WATER REQUIREMENT OF CORN (HOGUE)
AVERAGE FOR 3 YEARS, 1910, 1913 1914

Condition of Soil	Soil saturation	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
					Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
	Pct.		Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Too dry	50	15	81	1057	165	379	105.5	695	289
Favorable	70	16	107	1249	230	522	162.2	716	316
Too wet	95	16	103	1090	168	422	142.1	854	341

[1] Average of ratios.

falling-off in production was accompanied by 12 per cent less total transpiration, 19 per cent increase in water used per unit of grain production, and 8 per cent increased transpiration per unit dry matter.

It would seem that these water-requirement effects cannot be interpreted as control acceleration or retardation of the transpiration rate as such, but rather are merely accompanying results of differences in the plant size and thrift of growth.

Combined moisture and fertility effects. Following the theory that increased fertility would reduce the water-requirement of crops on soils where moisture shortage is a decidedly limiting factor in production, a series of tests combining variations in soil moisture and soil fertility were made in 1915 and 1916 (Table 15). These consisted of growing Hogue Yellow Dent corn on both manured and unmanured surface soil maintained at the varying degrees of 40, 50, and 70 per cent relative soil saturation.

TABLE 15. EFFECT OF MANURE UPON THE WATER REQUIREMENT OF CORN (HOGUE) GROWING IN SOIL OF VARIOUS MOISTURE CONTENTS
TWO-YEAR AVERAGE, 1915–1916

Soil Treatment	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter
		Ins.	Sq.in.	Gms.	Gms.	Kgms.	Gms.	Gms.
40 per cent soil saturation								
Without manure	7	87	791	115	256	48.3	438	197
With manure	7	89	967	133	314	59.3	452	194
50 per cent soil saturation								
Without manure	8	107	963	167	372	72.6	433	195
With manure	8	104	1067	170	394	77.7	460	202
70 per cent soil saturation								
Without manure	8	109	1070	174	425	95.5	549	224
With manure	7	111	1125	212	489	105.4	495	216

[1] Average of ratios.

The 40 and 50 per cent relative saturations proved sufficiently dry to reduce the total production 40 and 10 per cent, respectively, below that of the 70 per cent. Application of sheep manure at the rate of 13 tons (moisture-free) per acre increased the total transpiration per plant 23, 7, and 10 per cent respectively for the 40, 50, and 70 per cent saturations, while it had no material effect upon the water-requirement ratio. It should be borne in mind when interpreting these results that a productive soil was used.

The increase in the amount of water used per plant and the slight effect upon the water-requirement ratio fail to indicate that manure application to a fertile soil under droughty conditions will serve to economize in the use of water.

SEASONAL VARIATIONS IN THE WATER REQUIREMENTS OF CORN

The transpiration relationships of Hogue corn were determined under similar cropping conditions throughout the 14-year period of 1910–1923. Potometers of standard size were filled each year with an average of 245 pounds (moisture-

free) of surface soil taken from the Experiment Station farm. Water was supplied in favorable quantity by the subirrigation method. The results for the various years are ranked in Table 16 in accordance with the magnitude of the water-requirement ratio.

TABLE 16. SEASONAL VARIATIONS IN THE WATER REQUIREMENT OF CORN (HOGUE) AND THE EVAPORATION FROM A FREE WATER SURFACE

Year	Total no. of pots	Dry matter		Leaf area per plant	Transpiration per				Evaporation free-water surface 36 sq. in. area
		Grain	Total		Plant	Gram grain	Gram dry matter	Sq.in. leaf area	
		Gms.	Gms.	Sq. in.	Kgms.	Gms.	Gms.	Gms.	Gms.
1915	12	214	518	1416	99.6	467	192	71	172
1922	39	200	506	1269	110.2	526	218	87	296
1921	33	218	488	1199	116.8	537	239	97	252
1917	12	186	416	1072	102.8	553	247	96	287
1916	12	204	514	1255	132.0	647	257	105	291
1912	48	199	407	805	106.0	533	260	132	351
1910	4	201	484	1313	127.0	632	262	97	283
1920	16	195	418	1156	110.4	566	264	96	287
1923	6	244	479	1127	127.6	522	266	113	281
1914	12	231	519	1279	142.1	615	274	111	284
1918	12	174	434	1027	136.1	782	314	133	328
1919	16	142	338	889	113.8	801	337	128	376
1911	4	179	413	890	144.0	804	349	162	391
1913	80	147	372	1071	165.0	1122	444	154	476

With some irregularity, considerable correlation is seen for the different years between the transpiration per gram total dry matter, per gram grain, per square inch leaf area, and the evaporation rate from a free water surface. All of these transpiration ratios and the free-water evaporation were minimum in 1915 and maximum in 1913. To illustrate, in 1915 and 1913 (1) the respective transpiration per plant was 99.6 and 165 kilograms; (2) the transpiration per gram total dry matter, 192 and 444 grams; (3) the transpiration per gram grain, 467 and 1122 grams; (4) the transpiration per square inch leaf area, 71 and 154 grams; and (5) the evaporation from a free water surface of 36 square inches area, 172 and 476 grams. Under field conditions (Table 17) the yield of corn at the Experiment Station averaged 75 bushels per acre in 1915 and 8 bushels in 1913. Comparing 1913 with 1915, the temperature during July and August, when the bulk of the water is normally used by the crop, was 13°F. higher, the relative humidity 26 per cent lower, the wind velocity 2 miles per hour higher, the possible sunshine 15 per cent higher, and the annual rainfall 7 inches lower. These climatic differences largely account for the differences in transpiration. The total annual rainfall is not indicative of the favorableness of the moisture supply, since this is modified by such factors as the soil-moisture carry-over from the previous year, the character of the soil and cultural practices, and the distribution of the precipitation.

It appears that, in general, in years of least available moisture during the growing season the atmospheric demand for transpiration is greatest. Thus a twofold hardship is experienced by plants growing under drouthy conditions—abnormal

TABLE 17. SEASONAL CLIMATIC EFFECTS UPON THE WATER REQUIREMENT OF CORN (HOGUE) AND THE EVAPORATION RATE FROM A FREE WATER SURFACE

Year	Transpiration per		Evap. free-water surface 36 sq. in. area	Grain yield per acre in field	Climatic factors				
	Plant	Gram dry matter			Air temp.	Rel. humidity	Hourly wind velocity	Possible sunshine	Yearly rainfall
	Kgms.	Gms.	Gms.	Bus.	°F.	Pct.	Miles	Pct.	Ins.
1915	99.6	192	172	75	74	67	8.8	67	34.7
1922	110.2	218	296	47	80	57	8.3	63	24.8
1921	116.8	239	252	66	82	63	8.8	59	25.9
1917	102.8	247	287	47	81	56	9.8	75	23.2
1916	132.0	257	291	62	84	54	9.7	77	22.5
1912	106.0	260	351	48	84	51	11.1	77	24.4
1910	127.0	262	283	58	80	53	9.3	71	28.0
1920	110.4	264	287	54	79	55	8.0	71	28.9
1923	127.6	266	281	61	79	59	8.7	65	29.0
1914	142.1	274	284	53	84	59	9.1	71	36.4
1918	136.1	314	328	3	84	52	9.9	62	22.4
1919	113.8	337	376	21	84	53	10.1	75	31.5
1911	144.0	349	391	43	82	51	10.8	81	24.1
1913	165.0	444	476	8	87	41	10.8	82	27.7

moisture shortage and abnormal atmospheric demand for transpiration. It would seem best in farm practice to adjust the amount of vegetation grown per acre through plant type and seeding rate to meet the average seasonal climatic conditions.

EFFECT OF WEEDS

As suggested by the data in Table 18, weeds use water in their growth at the expense of the crop, and reasonable means should be employed in their control. During a 7-year period wild sunflowers (*Helianthus annuus*), such as are frequently found in corn fields, were compared with Hogue Yellow Dent corn as to their transpiration relationships. The corn and sunflowers used respectively 126 and 322 kilograms water per plant, while their water-requirement ratios were respectively 269 and 561.

TABLE 18. RELATIVE WATER REQUIREMENTS OF CORN AND WILD SUNFLOWER AVERAGE FOR 7 YEARS, 1914–1920

Description	Total no. of pots	Dry matter		Transpiration per		
		Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Gms.	Gms.	Kgms.	Gms.	Gms.
Hogue Yellow Dent corn	56	196	476	126.2	657	269
Sunflower (*Helianthus annuus*)	28	76	581	322.0	4606	561

[1] Average of ratios.

EFFECT OF VARIATIONS IN METHODS UPON THE WATER REQUIREMENT OF CORN

In a study of potometer technique in connection with these transpiration investigations several variable factors were introduced, including pot size, number of plants per pot, stage of maturity, and exposure of pots.

It is apparent from the data in Tables 19–22 that overcropping of the soil either by the use of too small pots or by growing too many plants per pot should be avoided. Data applicable to field conditions should be secured under as nearly normal field exposure as can be arranged, and mature plants supply the most significant results.

TABLE 19. RELATION OF POT SIZE TO THE WATER REQUIREMENT OF CORN (HOGUE) AVERAGE FOR 2 YEARS, 1914–1915

Size of pot	Manure	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
					Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
Ins.			Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
12×12	No	8	74	729	9	104	31.5	3775	310
	Yes	8	91	972	43	189	54.7	1369	266
12×24	No	8	99	1113	39	185	55.8	1472	294
	Yes	8	104	1203	92	287	81.0	1133	264
16×24	No	8	110	1247	81	282	81.7	989	283
	Yes	8	112	1314	148	405	113.7	857	270
16×36	No	7	115	1201	175	413	99.4	562	238
	Yes	12	113	1294	206	597	120.8	578	235
21×36	No	8	114	1340	272	656	150.3	577	232
	Yes	8	117	1364	263	671	155.5	583	229
30×36	No	7	112	1248	284	677	145.7	512	213
	Yes	8	113	1297	314	733	168.0	514	220

[1] Average of ratios.

TABLE 20. EFFECT OF THE NUMBER OF PLANTS GROWN PER POT UPON THE WATER REQUIREMENT OF CORN (HOGUE) SEVEN-YEAR AVERAGE, 1914–1920

Plants per pot[2]	Total no. of pots	Plant height	Leaf area per pot	Leaf area per gram dry matter	Dry matter per pot		Transpiration per		
					Grain	Total	Pot	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq. in.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
1	48	101	1176	2.5	196	476	126.2	657	269
3	24	93	3020	5.5	145	563	152.3	1137	275
6	24	82	4574	8.0	71	584	159.2	2607	290

[1] Average of ratios.
[2] All pots 16″×36″.

TABLE 21. COMPARATIVE WATER REQUIREMENTS OF CORN (HOGUE) GROWN TO SILKING STAGE AND TO MATURITY AVERAGE FOR 6 YEARS, 1913, 1915–1917, 1919–1920

When Harvested	Total no. of pots	Plant height	Leaf area per plant	Total dry matter	Transpiration per		
					Plant	Gram dry matter[1]	Square inch leaf area[1]
		Ins.	Sq. in.	Gms.	Kgms.	Gms.	Gms.
Silking stage................	24	96	1213	250	70.4	296	59
Mature....................	48	97	1152	453	125.7	284	112

[1] Average of ratios.

TABLE 22. RELATION OF THE EXPOSURE OF THE POTOMETER TO THE WATER REQUIREMENT OF CORN (HOGUE YELLOW DENT) AVERAGE FOR 6 YEARS, 1914–1919

Exposure of Potometers	Total no. of pots	Plant height	Leaf area per plant	Dry matter		Transpiration per		
				Grain	Total	Plant	Gram grain[1]	Gram dry matter[1]
		Ins.	Sq. in.	Gms.	Gms.	Kgms.	Gms.	Gms.
Surrounded by corn field								
Buried in soil	43	98	1180	164	423	110.5	689	261
Water jacket	24	96	1140	179	450	112.7	649	250
Exposed in pit	48	101	1179	196	482	127.9	667	270
Standing in open								
On ground	40	88	971	131	372	123.2	1045	340

[1] Average of ratios.

Conclusions

Transpiration is essentially evaporation. The plant exercises no important vital control over its transpiration rate. The amount of water used by any specific crop depends largely upon the amount of functioning vegetation and upon the intensity and duration of atmospheric demand for moisture as measured by free-water evaporation. The vegetative development varies with the plant type, the seeding rate, and the favorableness of growth conditions—including soil productivity, soil moisture, and climatic factors. In farm practice many of these factors, exclusive of climate, can be advantageously regulated to meet the local conditions. Small plant size, early maturity, and relatively thin spacing are conducive to low-water consumption per unit area of land.

The water-requirement ratio does not appear to be a very important index as to the relative suitability of various crops to any particular region or set of conditions. There doubtless are other more important physiological factors and economic reasons for relative excellence and choice of crops. In the state of Nebraska where water shortage is nearly always a limiting factor in production, the respective annual acreages of corn, wheat, and oats approximate 8,000,000, 3,500,000, and 2,500,000, whereas the comparative water-requirement ratios during a 2-year period were 230, 321, and 426. One and a fourth million acres of alfalfa are grown annually, and the acreage should be doubled, although the water-requirement ratio of this crop is fully 3 times that of corn.

These water-requirement ratios for the various crops tested appear to have some significance in indicating relative crop adaptation from the standpoint of total tonnage produced. As an average for 9 years, 1916–1924, corn, winter wheat, and oats grown on the check plats of the field fertilizer experiments, have yielded 5451, 5178, and 3029 pounds air-dry matter per acre, whereas the respective water-requirement ratios in a 2-year potometer test averaged 230, 321, and 426. With its very high water-requirement ratio, alfalfa cannot be regarded physiologically as a specially drought-resistant crop. In upland fields

this crop tends to become relatively unproductive after a period of years, doubtless due to depletion of the subsoil moisture upon which it draws to a far greater depth than any other field crop.

Comparing different varieties within a crop, such as corn, those which are recognized as productive sorts and which mature satisfactorily have reasonably similar water-requirement ratios. No variety of corn was found with an outstandingly low water-requirement ratio. It appears that varieties acclimated to humid conditions may have no inherently greater water-requirement ratio than those acclimated to semi-arid conditions.

Sorghum varieties which are recognized for special drought resistance in the Great Plains area of Kansas, Oklahoma, and Texas, had no lower water-requirement ratio than corn. Their acknowledged superior drought resistance in comparison with that of corn was not indicated by the water-requirement ratio and is doubtless due to other physiological differences. It is suggested that these crops may differ somewhat in their suitability to droughty conditions by virtue of various differences: (1) Extent of root system, (2) osmotic pressure of the cell sap, (3) ability of the plant to enter into a relatively dormant state during periods of drought, (4) sorghum possesses a tendency to produce grain-bearing branches if the main head fails to materialize, and (5) there is less likelihood of non-fertilization with sorghum than with corn in the case of severe drought at the pollination period.

Increasing the fertility of soil up to maximum production under the prevailing conditions is commonly accompanied by a lowering of the water-requirement ratio. It appears that this cannot be attributed to transpiration regulation on the part of the plant because actually more water is transpired per plant, due to greater vegetative growth. The lowering of the water-requirement ratio is merely an incidental accompanying result of more thrifty and productive growth without a proportional increase in the amount of water consumption.

The theory that regions now too dry for crop production can be made productive through an increase in soil fertility, thereby increasing the concentration of the soil solution and permitting the intake of the necessary amount of solutes with less water, is not in agreement with facts. Crop growers in semi-arid regions are justifiably cautious in the use of barnyard manure, commercial fertilizers, and legume rotations for fear of "burning up the crops."

There seems to be no practical way of controlling constructively the water-requirement ratio through regulation of the water supply, aside from providing more favorable conditions of growth. Excessive soil moisture increases the water-requirement ratio as a result of reducing the yield without proportionately reducing the vegetative size.

Seasonal climatic differences influence the water-requirement ratio more than ordinary variations in soil, moisture, culture, or variety. Other conditions being equal, it varies from year to year much as does the evaporation from a free-water surface.

Assuming a yield of $2\frac{1}{2}$ tons dry matter per acre and assigning the 14-year

KIESSELBACH: WATER REQUIREMENTS OF CROPS

average water-requirement ratio for corn of 277, we have a total transpiration of 692 tons per acre, or 6.3 acre inches.

If under field conditions we assume a stand of 2 plants per hill in hills 42 inches apart, and assign the 14-year average potometer requirement of 273 pounds of water per plant, a total usage of 971 tons per acre is indicated, which is equivalent to 8.8 acre inches. Such a stand, yielding at the potometer rate of 450 grams dry matter per plant, would produce 3.5 tons dry matter per acre.

The average annual transpiration per acre of corn in eastern Nebraska probably falls between 6.5 and 9 acre inches, which amount fluctuates greatly in different years. The average annual precipitation in this region is approximately 28 inches, two-thirds or more of which is lost by other means than by transpiration from the corn crop.

When the atmospheric demand for transpiration exceeds the supply of water readily available to the plant, the crop is likely to undergo a set-back resulting in reduced yields. Cultural practices should therefore be followed which tend to conserve the moisture. Such practices are designed primarily to prevent run-off, weed growth, and over-cropping. Well-adapted crops should be grown. This results in a relatively low water-requirement ratio. Caution should be exercised to avoid excessive fertilization of the soil under conditions of moisture shortage, because high fertility is conducive to large vegetative development and consequent high water consumption per plant.

Increased soil fertility may often be advantageously utilized with some crops under relatively dry conditions through seeding or other cultural practices which counteract somewhat the vegetative stimulation. In the case of corn, for example, this may consist in the use of a somewhat smaller variety, listing, or wider spacing.

EXPERIMENTAL ERROR IN FIELD PLOT TESTS[1]

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As a method of investigating problems of crop production, the field experiment is subject to two distinct types of error. The experiment is essentially a determination of the differences which result when one (or more than one) factor in crop production is varied while all other factors remain constant. Thus, in a variety test, plots of uniform soil are seeded in a uniform manner to different varieties, and the differences in their yields are taken to measure the relative value of the varieties for the conditions of the experiment. Any variation in the factors not under investigation (in this case the soil, the cultural conditions, etc.) leads to experimental error, for it causes differences in the yield of the plots which may not fairly be attributed to the variety. But in addition to this type of error, which is common to all comparative experiments, the field experiment is subject to error of another sort. Its chief purpose—usually its only purpose—is to measure effects which may be expected in actual field practice. Now the effect of almost any factor in crop production varies greatly under different conditions. The best variety of corn on a rich soil may be only mediocre on a poor soil, the best variety of oats under early seeding may be relatively poor under late seeding; the best variety of wheat in an experimental field with a thoroughly prepared seed bed may winterkill much more than others under ordinary farm conditions. If the conditions of the experimental field differ materially from field conditions in the region in which the results are applied, the results of the most accurate experiment may be very misleading as a guide to farm practice. The errors of application thus introduced are usually not diminished by refinements of technic designed to reduce the experimental error; in fact the reverse frequently occurs, for these refinements often make the conditions of the experiment less like those of the field.

Both types of error are involved in every field experiment, for the conditions of the experiment can never be either perfectly uniform or perfectly representative. Probably errors of application are the more important as a source of false conclusions, but it is experimental error which has received by far the greater share of attention and investigation. We now have a fair working knowledge of the experimental error which results from variation in the soil of the experimental field, but we know very little of the error of application which results from differences between the soil of the experimental field and that of the farm

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on which the experimental results are applied. There is little to be gained by reducing the experimental error from 5 per cent to 3 per cent if errors of 25 per cent and 50 per cent are involved in the application of the results. The urgent need at present in the improvement of the field experiment is a more thorough study of the interrelations of factors in crop production, which will make possible a more rational application of experimental results.

In this paper I shall discuss only the experimental error, that is, the error which interferes with the correct determination of the results to be expected under the conditions of soil, climate, and culture of the experimental field. Experimental error results from variation in plants, cultural conditions, soils, and seasons within the experiment. Since all of these factors are incorrigibly variable, we must recognize at the start that it is impossible to determine exactly the relative value of the varieties or treatments compared, even for the single field on which the experiment is performed. All we may hope to discover is their approximate relative values, together with some estimate of the accuracy of the approximation.

Now what we are attempting to approximate, in the case of a test of 4 wheat varieties for example, is what each of the 4 varieties would yield if it alone had been grown on the entire experimental field through an infinite series of seasons. If we had this information for each of the varieties we would know their true relative yields on the field in question, with no experimental error whatever. Of course it is impossible to grow all of the varieties simultaneously on the entire field, or to continue the experiment through an infinite number of seasons. But each variety may be grown on a part of the field which will serve as a sample of the whole and from this sample we may estimate what the variety would have yielded if it had been grown on the entire field. Similarly the experiment may be continued through a series of seasons, which will serve as a sample of seasons in general in the locality. If the conditions of random sampling are satisfied, we may then estimate not only what each variety would have yielded on the entire field and through the entire period, but also the degree of precision in our estimate of these yields.

But it should be noted that we are concerned here with sampling 2 distinct populations, a population of plots and a population of seasons. When the only information required is the relative yields in a particular season or group of seasons, as may occasionally be the case, the experimental error is due entirely to plot variability, and seasonal variability may be disregarded. But ordinarily the object of the experiment is prediction for future seasons, and the results in the seasons covered by the experiment are of use only in so far as they indicate what may be expected in the unknown seasons to come. Ordinarily, therefore, the significance of the experiment is dependent not only on the probable error determined from the variability of the plots,—which indicates the precision with which the plot yields represent the yields to be expected from other series of plots taken at random from the same field during the season or seasons concerned—but it is dependent also on the probable error determined from the variability of the seasons, which indicates the precision with which the experi-

mental results represent those to be expected on the same field in other series of seasons taken at random. The 2 types of error are distinct and independent, and the measures taken to control either one have no effect upon the other. We shall therefore consider them separately.

Plot Variability

The extent and nature of plot variability may be most directly determined by performing a field experiment "in blank," that is, by determining the yields of all of the plots of the experimental field without differential treatment. It is also possible, from the data of the blank experiment, to determine by actual trial the effectiveness of various methods used to control the error from plot variability. Thus by combining adjacent plots in groups, larger plots of various sizes and shapes may be formed, by combining distributed plots replication may be simulated, and by using certain plots as checks the yields of the remaining plots may be adjusted in various ways. The effectiveness of each of these operations in controlling experimental error may be measured directly by determining the effect on the variability of the plots. These possibilities have made the blank experiment a popular means of investigation, and during the last 15 years many studies of this type have been reported.

The advantages of the blank experiment were, however, recognized in the early days of the field experiment. As early as 1849 Johnston pointed out as one of the principal sources of error in the field experiment "failure to determine the limits of variation in natural productivity of the field," although he gave no data of trials of this sort. Grouven in 1863 reported small blank experiments with wheat and rye. Drechsler in 1880 emphasized the value of such trials and urged that all fertilizer tests be preceded by a determination of the variability of the yields without fertilizer on the same field, and that in the case of standing experimental fields the preliminary test be continued for several seasons with different crops. The first extensive blank experiment was reported in 1897 by Larsen who harvested 960 plots of timothy without differential treatment. During recent years, a large number of similar experiments have been reported from different parts of the world, representing a wide diversity of crops, soils, and investigators. In Table 1 are listed 22 blank experiments, each including more than 100 plots, in which the yields are reported in full. These furnish material for the investigation of many questions of field plot technic, and for a general consideration of the nature of plot variability.

The Extent and Nature of Plot Variability

These blank experiments show clearly that the extent of plot variability may differ very widely in different experiments. It is sometimes said that the probable error of field experiments, except in the case of very small plots, is some relatively constant quantity, say about 5 per cent (Wood and Stratton '10). It is clear that so far as these blank experiments represent the conditions of experimental fields the true situation is very different. The probable error of a single

plot in percentage of the mean is about two-thirds of the coefficient of variability. Excluding the experiments on small plots we find a range in the probable error of the single plot yields from less than 5 per cent to more than 20 per cent. In fact, the blank experiments with small plots have not shown on the average as much plot variability as those with large plots, though there is no obvious relation between size of unit plot and variability. Nor does plot variability seem to be closely related to the crop, for with the same crop and even on the same field the variability may differ greatly in different seasons. All fields show some plot variability but plot variability itself is highly variable.

TABLE 1. BLANK EXPERIMENTS INCLUDING MORE THAN 100 PLOTS

Reference	Crop	Unit Plots		Coefficient of Variability	Coefficient of Heterogeneity (2×2 grouping)
		Size Sq. M.	Number		
Larsen 1897	Timothy	6.7	240	17.4[4]	.611[3]
Haritonenko 1907	Sugar beets	136.5	416	10.9[4]	.281[2]
Jegerow 1909	Oats	4.6	240	8.9[4]	.505[2]
Lehmann 1909	Millet 1905	404.8	103	16.3[3]	.735[3]
Lehmann 1909	Millet 1906	404.8	103	34.5[3]	.138[3]
Lehmann 1909	Millet 1907	404.8	103	29.3[3]	.716[3]
Smith 1910	Corn 1895	404.8	120	24.7[2]	.830[a3]
Smith 1910	Corn 1896	404.8	120	11.2[2]	.815[a3]
Smith 1910	Corn 1897	404.8	120	8.8[2]	.606[a3]
Mercer and Hall 1911	Wheat	8.1	500	11.6[1]	.336[3]
Mercer and Hall 1911	Mangolds	20.2	200	6.2[1]	.346[3]
Montgomery 1913	Wheat 1909	2.8	224	13.0[1]	.391[3]
Montgomery 1912	Wheat 1911	2.8	224	17.5[1]	.603[3]
Lyon 1912	Potatoes	20.2	204	22.3[2]	.311[3]
Tulaikow 1913	Spring wheat	4.6	240	12.8[4]	.334[2]
Tulaikow 1913	Winter wheat	4.6	240	9.9[4]	.353[2]
Kiesselbach 1918	Oats	134.9	207	7.8[1]	.495[b3]
Harris 1920	Alfalfa 1913 (2)	172.0	184	21.8[2]	.182[3]
Harris 1920	Alfalfa 1914 (1)	172.0	184	24.2[2]	.432[3]
Harris 1920	Alfalfa 1914 (2)	172.0	184	25.9[2]	.449[3]
Harris 1920	Corn 1915	172.0	184	13.5[2]	.498[3]
Harris 1920	Corn 1916	172.0	184	17.9[2]	.436[3]

[1] Author's data.
[2] Computed from author's data.
[3] After Harris, 1920.
[4] After Roemer, 1920
[a] Grouping 2×1
[b] Grouping 3×1

The use of the probable error in the interpretation of field experiments involves the assumption that the variations in plot yields on the experimental field approximately fit the normal curve of error. This assumption may be tested by means of the data from blank experiments. If the distribution of plot yields is normal, about 50 per cent of the plot yields will differ from the mean by no more than the probable error, half of these being greater and half less than the mean, and the proportions deviating from the mean by various multiples of the probable error may be determined from the tabled values of the probability integral. In Table 2 these values are shown in comparison with the percentages of plots actually found to give the corresponding deviations in the blank experiments.

TABLE 2. FREQUENCY DISTRIBUTION OF PLOT YIELDS IN BLANK EXPERIMENTS
PERCENTAGE OF PLOTS DEVIATING FROM MEAN BY AMOUNTS STATED

ɛxperiment	Number of plots	Deviation from Mean Divided by Probable Error													
		−7 to −8	−6 to −7	−5 to −6	−4 to −5	−3 to −4	−2 to −3	−1 to −2	0 to −1	0 to 1	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6
ʟarsen	240						5.4	19.2	32.1	21.7	12.1	6.3	2.9	0.4	
Haritonenko	416				0.5	4.8	6.0	14.2	17.8	30.8	20.0	5.8	0.2		
egerow	240				0.8	0.8	6.7	18.3	27.1	23.3	12.1	7.1	3.8		
mith 1895	120					3.3	3.3	13.3	36.7	22.5	13.3	2.5	2.5	2.5	
mith 1896	120	0.8	1.7	0.8	0.8	0.8	0.8	3.3	25.8	47.5	17.5				
mith 1897	120	0.8	1.7	0	0.8	0	1.7	9.2	25.8	42.5	15.0	2.5			
Montgomery 1909	224			0.9	0.9	1.3	3.6	18.8	26.8	21.9	17.9	4.9	2.2	0.4	0.4
Montgomery 1911	224				•	1.3	5.4	21.0	24.6	20.1	17.0	9.4	1.3		
ʟyon	204				0.5	2.0	2.9	17.2	30.4	20.6	20.1	3.4	2.5	0.5	
Tulaikow (spring)	240				1.3	0.8	6.7	14.2	27.9	25.0	16.7	5.8	0.8		0.8
Tulaikow (winter)	240				0.4	1.7	7.1	15.0	25.0	27.5	15.4	5.4	2.1	0.4	
Gorski-Stefaniow (sandy loam)	197					2.5	5.6	16.8	23.9	26.4	17.3	5.6	1.0	0.5	0.5
Gorski-Stefaniow (loess)	300					0.7	6.0	19.3	27.7	22.0	14.0	6.7	3.0	1.0	
Kiesselbach	207			0.5	0.5	2.4	5.8	14.0	20.8	31.4	18.8	5.3	0.5		
Harris 1913 (2)	184				0.5	2.7	4.9	19.0	27.7	17.9	19.0	4.9	3.3		
Harris 1914 (1)	184					1.6	4.3	21.2	26.6	25.5	10.9	7.1	2.2		0.5
Harris 1914 (2)	184					0.5	8.7	15.8	25.5	28.3	9.2	9.8	1.6	0.5	
Harris 1915	184						4.9	16.8	32.1	29.9	6.5	4.3	2.7	2.7	
Harris 1916	184					0.5	9.2	17.4	22.8	27.7	15.2	3.8	2.2	1.1	
Normal Distribution		.0+	.0+	0.03	0.31	1.8	6.7	16.1	25.0	25.0	16.1	6.7	1.8	0.31	0.03

The fit is by no means perfect, and in many cases the deviations are considerably greater than may be accounted for by error of sampling. For example, in the blank experiment with corn reported by Smith (1896 crop) about two-thirds of the plots yielded more than the mean and one-third less, instead of half and half; and almost three-fourths of the plots differed from the mean by less than the probable error, instead of the one-half expected in a normal distribution. The probable error of a single plot yield on this field, determined by the usual formula, is 6.92 bushels, but obviously the odds are not even that a single plot taken at random will differ from the mean by this amount or less; the odds are almost 3 to 1. These extreme results are caused by the inclusion of several "abnormal" plots which gave very low yields in 1896 and thus greatly increased the probable error. These plots may be eliminated when the field is used for a blank experiment, and the results will then agree more satisfactorily with expectation. But when the field is in ordinary experimental use the evidence is usually not sufficient to enable the investigator to eliminate "abnormal" plots.

In most of the blank experiments the distribution of plot yields approaches the normal much more closely, and on the whole the experiments indicate that variations in plot yield on experimental fields of the type used in these studies are fairly well represented by the normal curve. Although it must be remembered that in general these are selected fields, doubtless more uniform than the general run of fields used for plot experiments, it is probable that the assumption of normal variability in plot yields does not commonly involve any very serious error.

Another general feature of plot variability is the systematic variation in plot yields resulting from variations which affect similarly the yields of neighboring plots. This was noted by several investigators reporting blank experiments. Some of the fields reported vary fairly regularly in one direction or another but in many the most pronounced variations occur in irregular spots.

For a most convenient measure of the extent of this systematic variation we are indebted to Harris ('15) who proposed as a criterion of field heterogeneity the coefficient of correlation of neighboring plots. By the methods of intraclass correlation, for which Harris ('13) had previously developed greatly simplified formulae, it is possible to determine the correlation of plots in groups of any number and arrangement desired. The coefficient measures the precision with which the yield of a plot may be predicted from the known yield of any other plot of the same group. The coefficient determined will, of course, vary with the number and arrangement of the plots grouped, as well as with the area of the unit plot, since plots close together are likely to be more nearly similar than those farther apart on the same field. In comparing fields, therefore, it is necessary to use the same arrangement of plots in forming the groups.

It is important to distinguish clearly between heterogeneity and plot variability. These terms are often used interchangeably, and it is sometimes implied that heterogeneity is practically a measure of plot variability, or that it measures that part of the variability which is of importance in causing error. In fact the two conceptions are concerned with quite different qualities of the field. Plot variability, as measured by the coefficient of variation, refers to the extent of variation in plot yields; heterogeneity, as measured by the coefficient of correlation of neighboring plots, refers to the grouping of the variates. Two fields may differ greatly in heterogeneity and be equally variable, or they may differ greatly in variability and be equally heterogeneous. For example, suppose the plots of a certain field have a coefficient of variability of 10 per cent and a coefficient of heterogeneity of $r=0.50$. If on a diagram of the field the same yields were rearranged wholly at random, the heterogeneity would be reduced to about zero; if they were carefully classified and grouped the heterogeneity would be increased to almost 1.00. But regardless of the arrangement of the plots the variability of this series of yields always remains at 10 per cent. Similarly, by using arbitrary figures, 2 fields may be diagrammed with the same coefficient of heterogeneity and with coefficients of variation differing as greatly as is desired.

Nor is there any consistent relation between the variability and heterogeneity of the fields actually investigated. In Table 1 are shown the coefficients of heterogeneity and variation for all fields of more than 100 plots for which yield data have been reported. The most variable series of all, as it happens, has the lowest coefficient of heterogeneity. Other highly variable series, however, including the results of another season on the same field, show a high coefficient of heterogeneity. Relatively low heterogeneity coefficients occur with both high and low variability, and relatively low coefficients of variability with both high and low heterogeneity.

There is, however, a definite relation between plot variability and hetero-

geneity. The value of the heterogeneity coefficient is dependent upon the relation between the variability of the ultimate plots, or units, and that of the combination plots, or groups. Since for any given degree of variability of combination plots, the variability of the ultimate plots as a whole will be entirely dependent on the variability of units within the group, the coefficient of heterogeneity is dependent on the relation between the variability of groups and the variability of the units within them. If the plots making up each group were a random sample of the field as a whole, the variability of the groups would be equal to that of the units, divided by the square root of the number of units included in each group. When the coefficients of variation of the groups and units bear this relation to one another the coefficient of heterogeneity is zero. As the variability of the groups becomes greater (or as that of the units becomes less) the correlation increases, until it reaches its maximum value of 1.00 when the variability of the groups is equal to that of the units. This would occur on a field in which the groups differ in yield but the units within each group are equal. On the other hand when the variability of the groups is less than that of the units divided by the square root of the number per group, the coefficient has a negative value. This might conceivably occur when some factor like competition, affecting the yields of neighboring plots in opposite directions, outweighed in effect factors like soil variation which affect the yields of neighboring plots similarly. If the variability of the groups were reduced to zero a field of varying plots grouped by twos would give a perfect negative correlation, for the yield of each member of a pair would deviate from the mean exactly as much as its partner and always in the opposite direction. With plots combined in groups of more than 2, a perfect negative correlation is, of course, impossible. In actual practice no negative heterogeneity coefficients have been reported.

Thus, a given coefficient of heterogeneity does not mean that variability is necessarily either high or low and does not indicate directly the accuracy of individual plots as samples of the field. A given coefficient of variation, on the other hand, indicates definitely how well the yield of a single plot represents that of the field and how large a number of such plots taken at random would be necessary for any degree of precision required.

Size, Shape, and Replication of Plots

The principal object of most of the blank experiments reported was to determine by direct trial the relative value of plots of different sizes and shapes and of different numbers of replications. The general conclusions of these trials agree fairly well. Replication of plots reduces the variability approximately in proportion to the square root of the number of replications. Increasing size of plots reduces variability but not so greatly as the equivalent amount of replication. Long, narrow plots are usually less variable than short, broad ones of equal area. Occasionally, the variability of long plots running in one direction is greater than that of short plots, a result accounted for by the fact that the principal variations in productivity extend in the other direction.

The relative effectiveness of increased size and replication in reducing plot variability has varied considerably in the experiments reported, as shown in

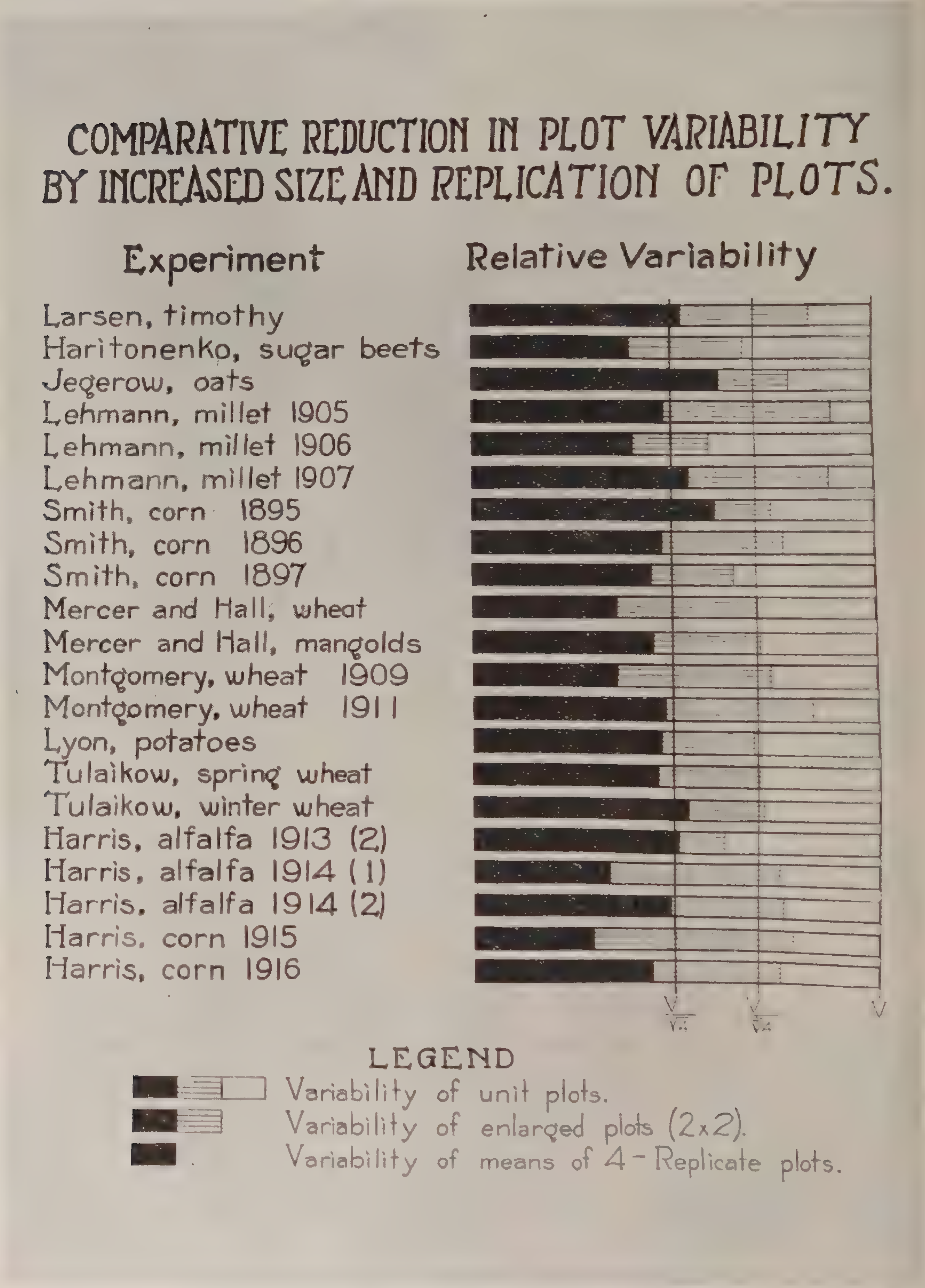

FIG. 1. Plot variability.

Figure 1. In all cases increasing size of plot is less effective than replication, but in some cases the difference is slight while in others it is much greater. Roemer ('20) who has used the results in 8 of these blank experiments, together with 3 smaller ones of his own, in the study of this question, has concluded that while replication reduces variability approximately in proportion to the square root of the number of plots grouped, increasing size of plot reduces variability approximately in proportion to the fourth root of this number.

When the nature of the plot variability is known the relative effectiveness of increasing size of plot and replication may be forecast. Increasing size of plot is equivalent to averaging adjacent plots, replication is equivalent to averaging systematically distributed plots. Averaging groups of plots taken at random would reduce the variability in proportion to the square root of the number of plots in each group. If the variations in yield were distributed at random over the field, averaging adjacent plots would have the same effect as averaging distributed plots, for in either case each group would be a random sample. But the yields are never distributed at random over the field, and neither groups of adjacent plots nor groups of systematically distributed plots form a random sample. We have seen that the yields of adjacent plots are usually positively correlated, and it is clear that the yields of systematically distributed plots may, through some relation to the systematic variations in productivity, be either positively or negatively correlated.

The relation of correlation to the variability of a sum is shown by the formula

$$\sigma_s=\sqrt{\sigma_1^2+\sigma_2^2+\cdots\sigma_n^2+2r_{1.2}\,\sigma_1\,\sigma_2+2r_{1.3}\,\sigma_1\,\sigma_3+\cdots 2r_{n-1.n}\,\sigma_{n-1}\,\sigma_n}$$

In this formula we may substitute for the standard deviations of the plots combined, σ_1, σ_2, etc., the standard deviation of the unit plots, σ_p, and for the correlations between the plots combined, $r_{1.2}$, $r_{1.3}$, etc., the coefficient of intraclass correlation for the grouping concerned, r. The standard deviation of the combination plot σ_c may then be computed from that of the unit plot σ_p by the formula

$$\sigma_c=\sigma_p\sqrt{n+n\ (n\text{-}1)\ r}$$

When this formula is used to determine the effect of increasing size of plot, r is the coefficient of heterogeneity for the corresponding grouping. For example, in Jegerow's blank experiment, in which the standard deviation of unit plots was 18.05 g, and the coefficient of heterogeneity for a 2×2 grouping was $r=0.505$, the standard deviation of plots of 4 times the unit size was $18.05\sqrt{4+4\times 3\times 0.505}=57.22$ g. The coefficient of variation was thus reduced from 8.89% to 7.05 per cent. This does not differ greatly from the value expected according to Roemer's rule ($8.89/\sqrt[4]{4}=6.29$) but it is obvious that when the coefficient of heterogeneity is near 0.75, as in Lehmann's 1905 experiment, or near 0.15, as in the same author's experiment of the following year, the effect of increasing size of plot may be very different. Increasing size of plot to 4 times the unit size decreases variability 10.5 per cent in the 1905 experiment and 40.5 per cent in the 1906 experiment. Since heterogeneity coefficients commonly vary over a wide range it is clear that no fixed relation such as that stated by Roemer will satisfactorily describe the results of increasing size of plot.

Similarly the relative variability of plots of different shapes is dependent on the correlation between the unit plots grouped in forming them.

Several investigators have found long plots in one direction materially less variable than in the other. Day ('20) found long plots running one way more variable and long plots running the other way less variable than short broad plots of equal area, and concluded "The results show conclusively that those plats having their greatest dimensions in the direction of least variation are more variable than plats that approximate squares in shape; and squares in turn are more variable than plats having their greatest dimension in the direction of greatest variation. . . . In an experimental area that was as variable in its length as in its width, the shape of the plat would exert no influence. When an investigator is unable to ascertain in which direction his soil is most variable, the use of square plats is probably advisable."

Since, as was noted above, the heterogeneity coefficient decreases as variation within the groups increases, the arrangement which groups the most variable unit plots will reduce variability the most. Ordinarily long narrow combination plots will include more variable units than short broad ones, since the units included are farther apart. If the variation in yield were regularly progressive from side to side of the field, and equally great in both directions, long plots in either direction would for this reason include more variable units than short broad plots and therefore long plots in either direction would be less variable than short plots. If the variation were much greater in one direction than the other, long plots in the direction of lesser variation might include less variable units than short plots, but this could occur only if the difference in variability in the 2 directions were extreme. Any tendency toward the spotted type of variability instead of the progressive type would decrease the variability of units within the short broad combinations and thus increase the advantage of long narrow plots. Long plots therefore are almost always less variable than short broad plots of the same area.

The reduction of plot variability by replication of plots will be approximately proportional to the square root of the number of replicates only if the correlation between the plots combined is approximately zero. It is usually stated that the results of actual trials agree with this, but the figures given sometimes deviate rather widely from expectation. On every field there is a very large number of possible systems of replication, and the result of the one trial made it but a random sample of the possible results. If the system used happens to fit the actual variations of the field in such a way as to bring together similarly yielding plots more often than random sampling would, the grouped plots will be positively correlated, and replication will be found to reduce variability less than expected. The reverse may also occur and variation may be reduced more than by random grouping. The latter is in fact the more frequent case, because the system of replication is often designed to make each group of replicates include representatives from the different parts of the field expected to differ most in yield. These irregularities may be avoided by substituting a

random method of choosing replicate plots, but this causes some inconvenience in planting and harvesting which may not be worth while.

The foregoing considerations apply to the question "What is the most efficient use of a given area for a given number of varieties or treatments?" The comparison in size of plot, for example, is between one plot of ¼ acre and 4 plots of 1/16 acre each. The evidence from blank experiments shows clearly that, so far as error from plot variability is concerned, a large number of small plots is more efficient than a small number of large plots covering the same area. The general opinion that large plots are more accurate than small ones is due largely to this evidence. But the evidence does not show that large plots are more accurate when the number of plots instead of the area is constant. By the method of comparison used increasing size of plot can hardly fail to decrease variability, for when two plots are combined the relative deviation cannot be increased and is almost certain to be decreased at least occasionally by the cancelling of a plus and minus deviation. If the unit plots of the blank experiment were each an acre in area, or any other size, this method of comparison would show that still larger plots are less variable. And so they are, so long as we are dealing with the same total area in both cases. But when increasing the size of plot increases the size of the field, as it must if we retain the same number of plots, additional soil variations are brought in, and the variability of the larger plots is increased. In comparing large and small plots on the basis of equal numbers the advantage of the large plot is therefore certain to be less than that indicated by the blank experiments, if indeed it retains any advantage at all. Similar considerations apply to replication, as various authors have noted, and though the evidence indicates that replication will reduce variability approximately in proportion to the square root of the number of replicates, when the same total area is concerned in both cases, we must expect the reduction in variability to be less than this when the same plot size is retained and the total area increased.

Adjustment of Yields

Since all experimental fields show systematic variations in productivity which affect groups of neighboring plots similarly, an obvious possibility of control is adjustment of yields. If the northwest quarter of the field is known to be 10 per cent higher in yield and the southeast quarter 10 per cent lower than the average, we may compensate for the inequality by decreasing all yields on the better quarter by 10/110 and increasing all those on the poorer quarter by 10/90. Clearly, if the productivity of each plot could be correctly estimated and its yield adjusted accordingly, all experimental error from variation in productivity could thus be eliminated. All methods of yield adjustment consist of these 2 steps or their equivalent: (1) Assigning to each plot a relative value (plot value) representing its assumed productivity in comparison with the average of the field, and (2) adjusting its actual yield to what it presumably would have been on an average plot. The effectiveness of adjustment in reducing experimental error is measured by the reduction in plot variability. Sometimes

the variability of the plot is increased by adjustment, and in this case the adjusted yields are less accurate than the actual yields and should not be used.

The chief difficulty is in determining the plot values. Obviously the yield of the plot itself may not be taken as an index of its productivity for purposes of adjustment—this is equivalent to taking the average of the yields actually obtained as absolutely correct and simply wiping out the variability. The productivity of each plot must be estimated either from its yields in preceding seasons or from the yields of neighboring plots in the same season. The former method (so-called "standardization" of plots) requires that a uniform crop be grown as a basis for an estimate of the relative values of the plots, and since these values differ widely in different seasons it provides a very unsafe basis for adjustment. The common method of adjustment in this country is to distribute uniform "check" plots over the field and from their yields estimate the productivity of the neighboring test plots. It is also possible to use the relative yields of neighboring test plots as a standard for estimating productivity, and thus to eliminate the check plots.

Various modifications of the check plot method are in use. Sometimes yields are expressed simply as plus or minus deviations from the yield of a nearby check. This is precisely equivalent to adjusting the yields by adding these deviations to the average check yield. Sometimes adjustment is made by ratio rather than addition. Probably the ratio method is more nearly correct, but unless the variations in productivity are large there is little difference in the results. The plot value assumed for a test plot may be determined by interpolation, on the assumption that the productivity varies uniformly from one check plot to the next. Objection is sometimes made to interpolation on the ground that the productivity does not actually vary uniformly. This is true, but neither does it vary by a sudden jump from that of one check plot to that of the next, as must be assumed if interpolation be rejected. Any method of assigning plot values involves some assumption as to the way in which productivity varies, and it is easy to show by assumed yields that any method may increase variability. The best method is the one which best fits the variations that actually occur; in other words, the one which in actual practice reduces plot variability the most.

Obviously the effectiveness of adjustment in reducing plot variability depends on the accuracy with which the plot values of the standard plots represent the relative yields of the neighboring test plots which are adjusted by them, in other words, on the extent of correlation in the productivity of neighboring plots. But plot variability is not necessarily reduced by adjustment, even though there is a considerable correlation.

For example, the relation of correlation to the variability of yields adjusted by ratio may be determined from the formula for the standard deviation of a series of indices, or quotients. The adjusted yield is the quotient obtained by dividing the actual yield by the plot value. The standard deviation of a quotient σ_q may be estimated by the formula

$$\sigma_q = \frac{m_1}{m_2}\sqrt{v_1^2 + v_2^2 - 2rv_1v_2}$$

in which m_1 and m_2 are the means and v_1 and v_2 the relative variability (σ/m expressed as a decimal fraction) of the dividend and divisor respectively, and r the correlation between them. The formula is approximate and applies only for the lower values of v_1 and v_2. For our purpose v_1 and v_2 are the variability of the actual yields and the plot values, which from the results of the blank experiments may be expected to be less than 0.35 ordinarily. Since the mean plot value is 1.00, and since v_1 and v_2 are practically equal, the relative variability of adjusted yields v_j in terms of that of actual yields v_a is approximately

$$v_j = v_a\sqrt{2-2r}$$

This means that the correlation of actual yields and plot values must be about $r=0.50$ to prevent an actual increase in variability by adjustment. With a correlation of $r=0.25$ adjustment will increase variability by about 22.5 per cent. A correlation of $r=0.70$ is required to decrease variability by this amount. On the fields for which heterogeneity coefficients have been given, adjustment of yields on the basis of one unit plot taken as a check in each 2×2 combination, would probably increase variability in most cases, for most of the heterogeneity coefficients for this grouping are below 0.50. In addition, on a field in ordinary experimental use, the reduction in replication necessitated by using one-fourth of the plots as check plots would increase plot variability about 13.5 per cent. It is clear, then, that adjustment of yields to be effective requires a method of determining plot values with some precision, and that in experiments in which adjustment is made the effect on variability should always be determined before the adjusted yields are used.

A modification of the method of yield adjustment recently suggested by Richey ('24) is a correction for regression in determining the plot values. Instead of assigning to the test plot a plot value deviating from the mean by the same amount as that of the corresponding check, the deviation of the check is multiplied by its regression coefficient. The result is a better approach to the true plot value and consequently a greater decrease in plot variability, though when the regression coefficient is near 1.00 the difference is of course negligible.

When the correction for regression is made, plot variability is reduced by adjustment according to the formula $v_j = v_a\sqrt{1-r^2}$ (Richey '24). Any series of plot values positively correlated with the actual yields will reduce variability. But even in this case, as Richey has pointed out, a high correlation is required if the reduction in variability is to be worth while. The relative effectiveness of adjustment with and without the correction for regression is shown in Table 3. The correction improves the effectiveness of adjustment in all cases, but unfortunately the improvement is greatest for low values of r, when adjustment by neither method is worth while, and for higher values of r the gain is small.

There is some experimental evidence on the effectiveness of various methods of yield adjustment. Standardization was suggested by Wagner ('80) who gave data showing a reduction in variability in small walled plots (Kastenparcellen) by this method, but rejected the method because of the difference in relative yields of plots which might be expected in other seasons. The blank experiments

of Lehmann were conducted primarily as a basis for the standardization of the plots. The yields of millet on the same plots for 3 seasons in these experiments,

TABLE 3. EFFECT OF ADJUSTMENT OF YIELDS ON PLOT VARIABILITY

Correlation of Actual Yields and Plot Values	Increase or Decrease in Coefficient of Variation	
	Adjustment Without Correction for Regression $v_j = v_a\sqrt{2-2r}$	Adjustment With Correction for Regression $v_j = v_a\sqrt{1-r^2}$
r	Per cent	Per cent
0	+ 41.4	− 0.0
.10	+ 34.1	− 0.5
.20	+ 26.5	− 2.0
.30	+ 18.3	− 3.6
.40	+ 9.5	− 8.3
.50	0	− 13.4
.60	− 10.6	− 20.0
.70	− 22.5	− 28.6
.80	− 36.8	− 40.0
.90	− 55.3	− 56.4
1.00	−100.0	−100.0

and those of corn for 3 seasons in the blank experiment reported by Smith, as well as their own extensive data on the yields of sugar beets, alfalfa, corn, oats, and barley on the same plots for 9 years, have been used by Harris and Scofield ('20) in investigating the inter-annual correlation of plot yields. Most of the correlations are positive and some are high, but in general they are well below $r = 0.50$ and several are negative. It is obvious from these correlation coefficients that the yield of plots in one season would be an extremely unsafe basis for adjusting the yields of another season.

Adjustment of yields by means of check plots was first proposed by Thorne ('90) of the Ohio station. Although a check plot method of adjustment was suggested in Europe by Larsen ('02), the use of check plots for adjustment has never become common except in America. The effectiveness of the practice has been determined in only a few of the blank experiments reported. In these cases it has usually been found to reduce variability slightly, but the common conclusion is that it is less effective than the equivalent amount of replication. It should be recognized also that even if adjustment were generally effective in blank experiments it would not be so necessarily in ordinary experimental practice, for though the yields of check plots may be closely correlated with those of neighboring plots of the same variety and treatment, they may differ considerably from the yields of these plots with another variety or treatment. Direct evidence is therefore needed to prove the efficacy of check plot adjustment, and this can be obtained only by the comparison of the variability of yields with and without adjustment in actual experiments. Some evidence of this sort is available, and indicates that adjustment often fails to reduce variability materially and even increases it in many cases, but that it may reduce variability much more than equivalent replication in other cases. This variation

in effectiveness is to be expected, not only because of the wide variation in the heterogeneity of different fields but also because some types of experiment are better adapted to adjustment by check than others. For example, the yields of pure lines of the same variety are more likely to be improved by adjustment to a check of the same variety than are the yields of widely different varieties, etc.

The disadvantages of adjustment by check are largely overcome when the yields of neighboring test plots are used as a standard for adjustment. Several methods for adjusting yields without check plots have been suggested. When the test plots are sufficiently replicated the yield of each test plot relative to the average of similar plots over the field is an approximate measure of productivity, and the plot value may be determined for each plot from the average of the relative yields of the group of plots of which it is the center. Moving average methods based on this principle were suggested by Hummel ('11) and Richey ('24). A method of adjustment without check plots, suggested by Mitscherlich ('12) is also equivalent to adjustment on a moving average. A different principle is involved in the method suggested by Surface and Pearl ('16) in which the plot values are those considered most probable on the theory of contingency. Detailed comparison and evaluation of these methods must be postponed for lack of space. Considered as a group in comparison with check plot methods they have several distinct advantages. Not only do they release the check plots for increased replication or other use, but they avoid the difficulty from differential response which may be involved when the reaction of a single variety or treatment is used as the sole basis for adjustment. Further, they permit the determination of each plot value from adjacent plots, which is not usually practicable in check plot methods, and they make it possible to base each plot value on a considerable number of standard plots, thus avoiding the disturbances that may be introduced by fluctuations of single check plots in adjustment to check. It is to be expected therefore that these methods, or other methods with similar advantages, will be more effective in controlling plot variability than the older methods of adjustment. Comparative studies of the effectiveness of different methods of adjustment in reducing plot variability in actual experiments are needed.

But adjustment to check has one noteworthy advantage. In many experiments the all-important comparison is between each variety or treatment tested and a certain standard variety or treatment. For example in a variety test there is usually one standard variety which any new variety must surpass to be of practical value. Adjustment to check is almost certain to increase the precision of the comparison of any tested variety with the check variety, no matter how it affects that of the comparison of tested varieties with one another. Adjustment is equivalent to determining for each tested variety its absolute or relative difference from the check in direct comparison. The probable error of the difference between any tested variety and the check, therefore, is simply the probable error of the adjusted yields of that variety. When yields are adjusted without checks the probable error of the difference between any variety and the

standard variety, like that of any other difference, must be computed from the usual formula, and will be larger than the probable error of the adjusted yields.

The method of pairing plots for comparison, lately much discussed under the name of "Student's method" is closely analogous to adjustment of yields. Basing the comparison of 2 varieties grown in adjacent plots on the series of differences between the paired plots is of course equivalent to regarding one of the varieties as a check and adjusting the yields of the other on the basis of their absolute deviations from the plot values established by the first. The effect, as in other methods of adjustment, is to eliminate that part of the variability which affects adjacent plots similarly. When a test of several varieties is conducted by comparing each with a standard in paired plots, any comparison between them must be made on the basis of their deviations from the standard or check variety. As in other methods of adjustment to check, the chief gain in precision is made in comparisons involving the check variety; other comparisons are improved only on the more heterogeneous fields.

But if the number of varieties is not extremely large this advantage may be extended to include all comparisons, by simply determining any required difference directly from the differences between plots paired as well as possible. In a test of 4 varieties, for example, if each series of 4 plots is arranged in a square, any of the 6 possible comparisons between varieties may be made directly from the differences between adjoining plots. When the number of varieties is larger, the plots paired for comparison will not always adjoin, but by pairing neighboring plots it will still be possible to eliminate some correlated variation. If the number of varieties is at all large the labor of computing the standard deviations of all possible comparisons is prohibitive. In a recent paper Student ('23) proposes a formula by which the average of these standard deviations may be very easily determined. This average may be taken as an approximate measure of the precision of a comparison of the mean yields of any 2 varieties in the experiment.

Seasonal Variability

Undoubtedly the cause of greatest difficulty in determining the relative value of varieties or treatments for a given field—in other words, the most important source of experimental error — is seasonal variability. Even though experimental error from plot variability were absolutely eliminated, the relative yields of the varieties or treatments tested would vary widely in different seasons, and the results of the most precise experiment in one season could give only a rough indication of the results to be expected in the next. Every agronomist recognizes this fact, and field experiments therefore are always continued through a series of several seasons before conclusions are drawn. But usually the extent of error in sampling seasons is not definitely measured, and no estimate is made of the likelihood that the results in future seasons would agree with those of the experiment. As a result conclusions regarding the relative value of varieties and treatments are often drawn from differences which from this standpoint are manifestly insignificant.

Experimental error from seasonal variability, like that from plot variability, is simply error of sampling. The difficulty in controlling the error is due to the fact that we do not know, and can hardly hope ever to know, as much about the extent and nature of seasonal variability as we now know about the extent and nature of plot variability. But we do know that seasonal variations in the relative yields of varieties and treatments do occur, and that the relative yields in the limited number of seasons included in an experiment are a random sample of these variations. Although the number of seasons covered by the experiments available is necessarily small, it should be possible by the use of methods adapted to small samples to gain some idea of the extent of error from seasonal variability in actual experiments. For this purpose we require data of field experiments in which the error due to plot variability is known and low, for otherwise the variation in yield in different seasons might be due in large part to plot variability.

During the 5-year period 1914–1918 inclusive, 77 varieties of wheat were tested in nursery plots at the Missouri station. Each variety was grown in 10 distributed single rod-row plots, sown and harvested by hand. The probable error of the mean yield of 10 rod-rows, computed in each season from the variability of the check plots, averaged less than 5 per cent. There were large and significant differences in yield in each season, but no varieties were eliminated before the end of the 5-year period. The mean yield of all varieties for the 5-year period was 25.5 bushels per acre. The mean yield in individual seasons varied from 12.6 bushels in 1917, a season characterized by severe winter injury, to 35.1 bushels in 1915. The average yields of the varieties for the 5-year period varied from 18.1 to 33.1 bushels per acre.

The so-called "probable error of the experiment," that is, the probable error of the mean yields of 5 years, computed from the probable errors of the results in individual seasons, was about half a bushel. This means that differences in average yield of more than about 2 bushels are significant as far as plot variability is concerned. In other words, if one variety outyielded another by more than 2 bushels per acre in this experiment, there is a strong probability that it is actually superior under the growing conditions of the 5 seasons in which the comparison was made, and if the experiment could be repeated under exactly the same conditions the first variety might with some confidence be expected to outyield the second.

But what we want to discover, if possible, is whether this variety will outyield the other in the long run of seasons. If the comparison could be continued indefinitely, the differences in yield of the 2 varieties would vary, sometimes favoring one and sometimes the other, sometimes being small and sometimes large. Our 5-year experiment has given us a random sample of 5 of these differences. The significance of the mean of the 5 differences, that is, the probability that it is not due merely to error in sampling the varying seasons, may be determined from Student's table.

There are 2,926 ways of pairing the 77 varieties for comparison, each of which gives a series of 5 differences between 2 varieties. The variability of these

series will differ considerably, not only because of the random fluctuations of the small samples, but also because some pairs of varieties react to seasonal variations more similarly than others. We cannot stop to consider these in detail, but we may get a general idea of the degree of variability from the average of the 2,926 standard deviations, which may be directly computed by Student's formula mentioned above. This average standard deviation is 7.1 bushels per acre. Considering odds of 30 to 1 ($P=0.9677$) as the lower limit of statistical significance, we find from Student's table that the least significant difference in the 5-year average yields is about 9.2 bushels per acre. Of the 77 varieties tested, all but 10 yielded within 9.2 bushels of the highest yield.

This extremely high standard deviation is of course due in part to plot variability, for if the conditions of the 5 seasons had been precisely the same, the annual differences would still have varied somewhat as a result of plot variability in each season. But the extent of plot variability in these experiments is known and is clearly too small to account for more than a small part of the variability found. The standard deviation of differences between varieties averaged about 2.3 bushels in the 5 seasons. The standard deviation measuring the extent of variation due purely to differences in season is therefore about 6.7 bushels ($\sqrt{7.1^2-2.3^2}$).

With so large an experimental error from seasonal variability, it is clear that some of the refinements of plot technic for the control of error from plot variability, which we have been discussing at such great length, would be wasted in this experiment. In the experiment as performed the error from plot variability is controlled sufficiently to make differences of 2 bushels in average yield significant, but it is unsafe to draw conclusions regarding the future performance of the varieties from differences four times as large. Perhaps it would be possible to reduce plot variability 25 per cent by adjustment of yields, for example, but this would only reduce the average variability of seasonal differences from 7.1 to 7.0 bushels, a negligible gain. If each variety had been grown in only 4 instead of 10 replicate plots in each season, plot variability would have been increased about 60 per cent, but the average standard deviation of differences would have been increased only from 7.1 to 7.7 bushels.

When we reflect that the low degree of significance which we have been discussing measures the precision of our estimate of the relative values of the varieties, not for ordinary field conditions but only for the conditions of the rod-row test, we are forced to the conclusion that this rather elaborate field experiment has yielded very little useful information. Undoubtedly its value would be somewhat greater if 3 or 4 replications of drilled 3-row plots occupying the same area had been substituted for the 10 replications of hand-seeded single rows, for experimental error would have been only slightly increased, and the final results would have applied to conditions much more similar to those of field practice. We have, in fact, since modified our nursery plot technic in this way.

But probably the error from seasonal variability is much smaller ordinarily than in this experiment. The variability of annual differences between varieties

is greatly increased by the different reaction of the hard and soft wheat varieties to the hard winter of 1917. The yields of practically all of the soft wheat varieties were greatly reduced in this season, while those of the hard wheat varieties were at least average. In the other seasons the soft wheat varieties in general outyielded the hard wheats. Consequently the elimination of this season would make the differences between hard and soft varieties much more consistent. There is no reason for eliminating this season from the records; such seasons are not rare under our conditions, and probably most random samples of 5 seasons would include at least a fairly hard winter. But some would not, and the experimental error in these would sometimes be much lower.

The inclusion of both hard and soft wheat varieties in this experiment is another reason for the high average variability. In experiments including varieties of only one type or the other, the seasonal variability of differences would be considerably lower, especially when the seasons included varied widely in adaptation to the 2 types, as in this case. When both types are included in the same test, if average standard deviations of differences are needed, they may be determined separately for comparisons between hard wheats, comparisons between soft wheats, and comparisons between hard and soft wheats. In the experiment discussed, the average standard deviation is decidedly smaller for comparisons within the type, but the differences between varieties of the same type are of course decidedly smaller also.

It is interesting to determine the extent of seasonal variability similarly in a test of pure lines of the same variety. In this sort of test we would expect a more uniform response to seasonal variations and consequently a lower average variability. No data of our own are nearly so complete and comprehensive as the records of the yields of 80 pure line selections of Turkey wheat during an 8-year period, recently published by Kiesselbach ('25). The yield tests in the first 3 seasons were in single rod-rows replicated 10 times, and in the following 5 seasons in blocks of 5 rod-rows, replicated 10 times. The average yield of the 80 strains for the 8-year period was 41.1 bushels per acre, the seasonal averages varying from 9.1 to 58.1 bushels, and the strain averages from 34.0 to 45.4 bushels. The probable errors of the mean of 10 plots, determined in each season from the variability of check plots, averaged 1.4 bushels, or 3.4 per cent of the average yield. The probable error of the 8-year average yields, measuring their error from plot variability, was about 0.6 bushel.

The average standard deviation of differences in this experiment, computed as before, is 4.6 bushels. The smallest difference in 8-year average yield of strains which may be considered significant from the standpoint of seasonal variability, is about 3.7 bushels.

The 8-year comparison is therefore adequate to show many significant differences in yield among these strains (although even in this exceptionally thorough and accurate test the 31 highest yielding strains do not differ significantly in yield). But testing pure lines for 8 years as a basis for elimination is a very rare practice in crop improvement. Usually a large proportion of the selected lines are eliminated for poor yield after 3 or 4 years' test, sometimes even after 2

years' test. A test of as much as 5 years' duration as a main basis of elimination is exceptional. No doubt this rapid elimination is often advisable, even though the odds involved are not as great as 30 to 1, but it is interesting to determine how great the risk of discarding the better lines would have been in this test if average yields for shorter periods had been used. Using as a basis the standard deviation determined from the 8-year test, the least significant difference in 5-year average yields is found to be 6.0 bushels, in 4-year average yields 7.6 bushels, and in 3-year average yields 12.2 bushels. Elimination of lines for poor yield after only 3 years test would appear to be a rather risky procedure. Even after 5 years' test there would be only a few strains which could be expected confidently to yield less than the others.

It might be supposed that the error from seasonal variability is greater in winter wheat than in spring sown crops, because of the wide variations in yield due to differences in winter injury. The variability of seasonal differences was determined from the yields of 5 varieties of corn which were included in the variety tests of the Illinois station throughout the 20-year period 1901–1920 inclusive, and those of 6 varieties of oats included in the variety tests of the Ohio station through the 20-year period 1893–1912 inclusive. These tests were in field plots and the extent of error from plot variability is not known. The average standard deviation of seasonal differences was 5.7 bushels in the corn variety test and 6.0 bushels in the oats test. Since the average yield in both experiments was about 61 bushels, the relative error from seasonal variability in these experiments is about equal to that in the wheat pure line test. In these 3 experiments the standard deviation measuring seasonal variation in varietal differences is in the neighborhood of 10 per cent of the mean yield, but it would be rash to assume without much more extensive investigation that this value will be approximated in other experiments. Probably the extent of seasonal variability will vary greatly in different types of experiment and in different localities.

But unquestionably experimental error from seasonal variability is a very serious limitation of the field experiment as a method of investigation. It may be possible, by thorough study of the reaction of varieties and treatments to climatic factors, to devise methods of reducing this error without great increase in repetition. There can be little doubt that, in the improvement of the field experiment, investigations of the extent and control of the error from seasonal variability are much more needed at present than further refinement of the methods for controlling the error from plot variability.

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THE RELATION BETWEEN PLANT GROWTH AND WATER TABLE ON DRAINED PEAT SOIL[1]

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Extensive studies on the relation between the water table and plant growth have been made in Germany (Bremen, Moorvers.-st.), Sweden (Jonkoping), United States (Minnesota, Wisconsin, and Michigan) and Russia. All of them, however, have studied the behavior of the water table only during the period of the year when crops were growing—the growing season. Very little attention was paid to the study of the relationship during the part of the year when no crops were grown—the dormant period. It is evident that during the dormant period the crops do not utilize the soil water. Nevertheless, the behavior of the water at this period must be of equal, if not greater, importance to the subsequent crops than the effect of the water table during the growing season. The reasons are as follows:

(1) Nearly all agricultural crops can be grown in water solutions, and the water as such has no harmful effect on the plant.

(2) There are 2 ways in which the water influences plant growth,—(a) directly, as a plant nutrient in a broad sense, and (b) indirectly, as a factor causing the leaching out of salts and a change in the soil structure. The latter effect, which will be explained in more detail later, seems to be most prominent during the dormant period (late autumn and early spring).

In other words, we have to follow the same method that is used in studying the lime problems, where we consider the direct effect of lime—as a plant nutrient—separately from its indirect effect, that is, the effect on the soil fertility.

It seems to me that lysimeter experiments are not suitable for our purpose, since it is difficult to maintain the same water condition in the lysimeter as in the field.

In studying peat soils as a medium for the cultivation of crops, I had to start the investigations with a study of the behavior of the water table and its relation to plant growth. The experiments were carried out in the field, in lysimeters and in pots. Only the results of the field experiments are discussed in this report.

The field experiment was carried out near Minsk, on the so-called "Komaroffskoe Boloto," a true peat bog of neutral soil reaction with 3 per cent of lime. The nature of the bog is described in more detail in one of my other papers.

After a preliminary survey a field plot of 4.5 acres, bordered by 2 parallel

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 18, 1926.

ditches, 252 feet apart, was chosen. The surface of the plot was almost level. The depth of the drainage ditches was 3.5 feet. At one end the depth of the peat layer was 3 feet, deepening toward the other end, where it reached 5 feet. The peat was underlaid by a fine sand mixed with granitic gravel. This made it easy for the water table to regain its former level after the frequent variations due to evaporation. Observation wells were located in the following positions:

No. 1 was placed at a distance of 7 feet from the ditch and the successive wells at a distance of 35 feet one from another, while the last one, No. 8, was again located at a distance of 7 feet from the second ditch. A second line of observation wells was located in a similar way, parallel to the first one. Both lines were perpendicular to the ditches. The first line of wells was placed in that part of the plot where the layer of peat was nearly 3½ feet deep, while the second line was placed where the depth of the peat was 5 feet. The wells were sunk 1.2 feet down into the mineral subsoil. The construction of the wells was carried out strictly according to the method of the Swedish Experimental Station (Jonkoping). The method of measurement of the water table itself was worked out by me; it made possible the measurement of the fluctuations of the table to the exactness of millimeters. I will not go into the details of this method but will just mention that in the computation of the data I used centimeters instead of millimeters, so as to eliminate the smaller fluctuations produced mainly by the changes in atmospheric pressure.

During all the growing season, from May 1, the observations were made every day, but before this time it was done only twice: first, the determination of the water level at the time when the snow had melted and when the upper 20 cms. in the middle of the experimental area were free from ice (March 26), and it was measured the second time on April 16, 1914. These measurements were made at the same points where later the observation wells were located, that is, in places corresponding to wells Nos. 1, 2, 3, and 4, as follows:

TABLE 1. THE HEIGHT OF THE WATER TABLE IN CM. PRIOR TO THE GROWING SEASON

Observation Wells	No. 1	No. 2	No. 3	No. 4
Water depth in cm. (Mar. 26)	32	14	10	3
Water depth in cm. (Apr. 16)	54	42	38	29

These figures show (1) that the drainage was very poor because the distance between the ditches was too great—252 feet; but it was made for the purpose of studying gradation in the plant development and in the water table on one single strip of peat soil area, without interruption by ditches; (2) the water level changes, traced from the ditches to the middle of the plot, are in the same order as is ordinarily indicated in all works on the subject; the water level is deeper on No. 1, at the ditches, and shallower in the middle, No. 4.

It was expected also that the water table later on would increase in height from the ditches toward the middle of the plot, where it would be at its maxi-

mum, and that during the driest part of the season this curve would approach a horizontal line.

The summer of 1914 was exceptionally dry. The precipitation during the growing period amounted only to 60 per cent of the normal and the vegetation around the place, especially on meadows and bogs, had suffered quite considerably from the drought. On peat soils, however, the vegetation was in very good condition.

On May 1 the water table showed a deviation from the normal curve and later on it began to change considerably, approaching a horizontal line. Table 2 shows in centimeters the average of the water table during the growing season from May 1 until September 1, given each half month.

TABLE 2. AVERAGE OF WATER TABLE, HEIGHT IN CENTIMETERS

Wells	May		June		July		August		Average
	I.	II.	I.	II.	I.	II.	I.	II.	
First Line of Wells									
1	62	55	71	59	65	54	55	55	59
2	60	52	69	54	62	51	51	51	56
3	66	58	75	59	67	55	55	56	61
4	70	62	79	63	71	59	59	60	65
5	70	61	79	62	71	59	59	59	65
6	63	55	74	58	66	52	53	53	59
7	61	53	71	57	65	52	52	53	58
8	63	55	73	60	66	54	55	55	60
Average	64	56	74	59	67	54	55	55	60
2nd Line of Wells									
9	71	66	83	69	80	68	69	69	72
10	68	61	77	62	72	67	68	69	68
11	67	60	77	59	77	68	68	69	68
12	67	60	77	56	76	67	69	70	68
13	67	58	77	54	75	66	67	68	66
14	68	61	80	58	78	68	69	70	69
15	67	59	79	59	78	67	67	69	68
16	71	61	82	63	77	65	66	67	69
Average	68	61	79	60	77	67	68	69	69

The water table did not show the expected rise in the middle of the plot but, on the contrary, in the first line it was even a little lower in the middle than at the ditches. The cause of the sinking of the water level in the middle below that of the area near the ditches deserves attention and should be thoroughly studied. However, at present, and for our purpose, it is enough to note that the water table was practically the same all over the experimental area, contrary to my expectations.

The crops used in the experiment were sown in uniform strips strictly parallel to the line of wells, as follows: (1) 3 varieties of oats (Nemerchansky, Seger, and a local variety); (2) pure species of grasses, such as *Festuca pratensis*, timothy, rye-grass, Westerwool-dicum, etc.; (3) mixtures of oats and vetch, and 2 grass

mixtures of Professor Weber (Bremen). Besides those in the experiment there were included some crops rarely used in cultivation, so that the cultivation could not have changed their character, such as *Phalaris arundinacea* L. and *Lotus uliginosus* Schk.

In the selection of the pure crops and mixtures I selected, as far as possible, all gradations of plants from those requiring much moisture to those requiring little. The same principle was applied in selecting oat varieties, the "Seger" being a variety requiring the largest amount of moisture, while Nemerchansky is supposed to be a variety requiring the minimum amount. The cultivation and fertilization with P_2O_5 and K_2O were careful and strictly uniform all over the plot.

Regardless of the nearly horizontal stand of the water table all over the experimental area it was easy to observe that the farther from the ditches toward the middle of the plot the smaller was the growth of the plants. A kind of zonation or gradation in the plant development was soon evident and became stronger every week. For the convenience of our studies the plot has been divided into 3 zones or belts, according to their location in relation to the ditches. These zones were parallel to the ditches. The first 7 feet from the ditches were not taken into account. The zone beginning with the seventh foot up to the forty-second is indicated as "border zone;" from the forty-second to the seventy-seventh, as "transition zone;" from the seventy-seventh to the one hundred twenty-seventh the "middle zone." The same designations were used for the other half of the plot.

The yield of every zone per square unit was estimated in per cent, taking the yield of the same square-unit of the middle zone as one hundred.

TABLE 3. COMPARATIVE YIELDS OF CROPS IN DIFFERENT ZONES

Crop	Middle Zone	Transition Zone	Border Zone
1. Oats, local variety	100	112	200
2. Beckmannia erusiformis	100	130	175
3. Timothy	100	140	175
4. Phalaris arundinacea	100	151	174
5. Festuca pratensis	100	153	169
6. Oats, var. Nemerchansky	100	112	158
7. Vetch and oats	100	135	158
8. Rye-grass Westerwool-dicum	100	130	152
9. Lotus uliginosus	100	122	145
10. Mixture of grasses No. 1	100	132	143
11. Oats—"Seger"	100	130	143
12. Mixture of grasses No. 2	100	125	138

Table 3 shows:

1. That all species and varieties within a species behave in the same way in respect to differences in growth; the maximum growth being in the "border zones" and the minimum growth in the "middle zone." This distribution corresponds to the behavior of the water table during the dormant period.

2. Those plants which are best adapted to grow in peat soil without drainage, as, for instance, *Lotus uliginosus*, show on the "border zones" a smaller increase

of yield than do the plants not adapted to such conditions. For example, oats showed on the "border zones" an increase in yield of 100 per cent while *Lotus uliginosus* showed only a 45 per cent increase.

3. The different varieties of the same species respond differently to the water table changes. This is well illustrated by the behavior of Swedish oats, "Seger," which gave an increase of only 43 per cent on the "border zone," while the common oats, the variety which requires well drained soil, showed an increase of 100 per cent.

Now the question is: What is the effect on yield of the water behavior during the dormant period of plant growth? To answer this question we must now study the water table during the early spring, March and April, and in the fall, after the growing season. The latter data are of significance only to characterize the changes of the water table, but not the yields, because such data are related to the season after the harvest.

Considering the same zones as were utilized above, Table 1 shows that in the early spring we have the changes of the water level in such direction that the zones of larger yields are those with the deeper water level. The average data of water level were:

TABLE 4. AVERAGE DEPTH OF WATER LEVEL IN SPRING AND DEPTH AT HIGHEST WATER POSITION IN FALL

	Spring		Fall	
	Depth in cm.	Relative Depth	Depth in cm.	Relative Depth
Middle zone	39	100	12.5	100
Transition zone	51	130	13.5	112
Border zone	72	185	23.2	186

Table No. 3 shows us that the yields in different zones lie between such limits: (a) the middle, 100; (b) the transition, 112–115; (c)border, 138–200.

It is evident that the changes of the water table during dormant periods correspond to the changes in plant growth. In other words, the changes in yield repeat those of water depth during the dormant period—in this case, changes during the early spring. It should be noted here that early spring and fall have the same water changes. I would like to remark here that my purpose now is to show only the tendency to the correspondence of these two lines in the changes; however, I can not formulate it precisely because of the difficulty of the determination of the fall beginning and the end of the dormant part of the spring. My purpose now is to show only the importance of the study of water table behavior during the dormant period.

Of great significance to plant growth is also the fluctuation of the water level during the dormant period. With different levels of water table there should also be differences in the colloidal behavior of the soil, which first of all affects the process of soil granulation. Ordinarily in the spring and fall, in the middle of the plot, the water is nearest to the soil surface and remains there longest, and the colloids which already have changed to jels might again return to the

state of sols. In this way the granular structure of the soil may again be dissolved. Near the ditches where the water level is lower we should find a granulated structure, the porosity of the soil increased, and the evaporation decreased. Table 5 shows the fluctuation of the water table in the observation wells; but the

TABLE 5. FLUCTUATIONS OF THE WATER TABLE, SPRING AND FALL

No. of Wells	1	2	3	4
Fluctuations in cm. (in the spring).......	9	16	19	19
Fluctuations in cm. (in the fall).........	23	36	43	46

fluctuations are still larger if we take the period from the beginning of spring until September 1 (Table 6). Here we have again a correlation of water fluctuation with the yield: the larger the yield the smaller the fluctuations, and in all instances, expressed relatively, we have nearly the same quantitative significance.

TABLE 6. FLUCTUATIONS OF THE WATER TABLE (FROM THE BEGINNING OF SPRING TO SEPT. 1).

No. of Wells	1	2	3	4
Fluctuations in cm....................	39	45	65	69

In Russia, as well as in the United States, I have observed the fact that a few days are sufficient to damage plant growth considerably, or destroy it entirely, if the water is allowed to remain near or above the surface of the peat soil.

During my last trip to the western part of the United States I noted near Stockton, California, that the fluctuations of the water table affected to a considerable extent the yield of potatoes under irrigation. This was especially evident on small farms where farmers try to use as much water as possible, to the extent that it sometimes stands over the surface. On the larger farms—usually better operated—the water table is not allowed to approach a height which might be harmful to the crops and, therefore, much larger yields are obtained in comparison with the smaller farms. The fluctuations of water table on small farms were observed to be about 20 cm. larger than those on large farms.

The largest fluctuations are observed in natural conditions where the water table is above the surface in the dormant period and in the summer sometimes one meter below the surface. It seems to me that the main task of peat soil drainage consists in diminishing these fluctuations of the water table as much as possible, and in the maintenance, at the same time, of a reasonable height of the water table.

The following conclusions may be drawn from the results of the experiments:

1. During the growing season the behavior of the water table has no influence on the plant development, under these conditions.

2. This development depends on the behavior of the water table during the dormant period. This relation is evident, from the study of crop development on zones running parallel to the ditches.

3. The water table has an indirect influence on the plant growth, depending on the following: (a) On the character of the peat decomposition and, especially, of the rate of the oxidation processes. (It seems that the oxidation of peat proceeds most rapidly in the early spring and early fall.) (b) On the leaching out from soil of easily soluble nutrients as a result of weathering. The oxidation and leaching processes are determined by water fluctuations. Therefore, the study of the influence of the water table must be directed toward the estimation of these fluctuations. These fluctuations determine also the formation of the granular structure of the soil and, consequently, its aeration, moisture, and temperature. In other words, to understand the relation between the water table and the plant growth, we must study the influence of the water table on the physico-chemical processes in the soil.

4. The exceptionally dry summer of 1914 offered a favorable opportunity to detect the relationship between plant growth and the influence of the water table during the dormant period. This was possible since the water table was nearly horizontal during the summer. A year with normal precipitation would have obscured this relationship.

5. It is evident that the study of the water table in swamps, bogs, and meadows during the growing season alone, as is usually done, does not give us a sufficient knowledge about the relationship. The relation between the plant growth and water table can be found only by studying it for the whole year, and in terms of those physico-chemical processes which are the results of the behavior of the water table.

BREEDING DISEASE RESISTANT VARIETIES OF CROP PLANTS[1]

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The application of Mendelian principles to plant breeding has led to a logical attack upon the problem of obtaining improved varieties. A greater interest in plant breeding has resulted and more knowledge of the reasons for the success or failure of certain crop varieties has been obtained. As one result of the studies of crop improvement it has been learned that the success or failure of a crop is dependent often upon its manner of reaction to a particular pathogene. At the present time the importance of disease resistant varieties of crops is recognized rather generally.

The science of genetics is generally considered to be the foundation upon which plant or animal breeding is dependent. In problems of disease resistance, however, other sciences are involved. In most breeding problems it is essential to grow the experimental crop under normal conditions for the purpose of isolating the better sorts. In breeding for resistance to pathogenic diseases the organism which causes the disease must receive as much attention as the crop itself and some means of creating an artificial epidemic of the disease must be found. Besides the genetic factors for resistance or susceptibility carried in the host plant there may be genetic factors in the pathogene which must be considered. These various phases of the problem of disease resistance have led some to raise the question as to whose job it was to produce disease resistant varieties (Jones, '26). There will be no attempt here to decide this question. The field is extensive, the problems are many, and there is room for all. Because of the nature of the problem it appears that there is an opportunity for a cooperative attack on disease resistance with the geneticist and pathologist in the major roles. Such cooperative studies have been under way in Minnesota for slightly more than 10 years. The present paper will consider chiefly the genetic phases of disease resistance and the closely related breeding problems, although the nature of the pathogenic organisms worked with will receive some attention.

Certain information regarding the parasite which causes the disease in question is of primary importance to the breeder. Obviously the constancy of the type of reaction between parasite and host plant is of major importance. Several

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years ago there was some doubt in the minds of many investigators as to the value of creating disease resistant varieties. It frequently happened that a variety which appeared resistant one year was rather highly susceptible in a later trial. This was considered rather generally to be the result of a change in the virulence of the parasite and some explained the results on the basis of a change induced in the parasite by a different host plant. Thus Pole Evans ('11) speaks of a hybrid between resistant and susceptible strains of wheat which caused such a change in the virulence of the parasite that after living on the hybrid the resistant parent became susceptible to the parasite and the susceptible parent became more susceptible. Plant species that supposedly modified the virulence of the parasite were considered as bridging hosts. An explanation of these phenomena that is in agreement with modern genetic principles has been obtained. Extensive studies conducted primarily by pathologists have proved that many disease organisms are composed of numerous strains or physiologic races which are rather similar morphologically but which differ widely in their parasitic capabilities. The development of this viewpoint has been gradual. It appears to be a natural viewpoint in the light of modern genetic principles. Numerous strains or physiologic races of many disease organisms have been found. This complicates the problem of obtaining desirable disease resistant varieties but gives added confidence that it can be solved. There is some experimental evidence for the conclusion that the individual physiologic races are as constant as higher plants. In studying the genetic phases of disease resistance the individual physiologic races of the parasite must be used. Certain phases of cooperative breeding studies at Minnesota will be discussed with particular attention to flax wilt, black stem rust of wheat and oats, smut resistance of oats, and the reaction to *Helminthosporium sativum*, the organism which causes the "spot blotch" disease of barley.

Wilt Resistance in Flax

Bolley ('08, '12) pointed out the value of creating disease resistant varieties and the growing of these as one means of controlling diseases. In his experiments with flax wilt, caused by *Fusarium Lini*, Bolley proved conclusively that varieties could be produced which in the presence of the organism were highly resistant.

Stakman, over fifteen years ago, repeated Bolley's experiments with flax, and as a result of these studies (Stakman, et al., '19) resistant varieties have been obtained. In these studies a specially prepared disease garden has been maintained and plants selected which appeared resistant. Tisdale ('16, '17) learned that high temperatures are favorable for the development of the organism and under such conditions even the most resistant varieties may wilt severely. He concluded that resistance and susceptibility were relative characters and dependent upon multiple factors.

Bolley was unable to explain why resistant varieties could be gradually developed under disease infection conditions. He believed, apparently, that resistance increased gradually as a result of long association between the parasite and the host plant. He also believed that resistant varieties gradually lost

their resistance when grown on wilt-free soil. Barker ('23) studied some of these questions at Minnesota. He concluded that the growing of flax on "sick" soil merely isolated the resistant strains and eliminated susceptible ones. Some flax varieties contained no resistant genotypes, whereas resistant genotypes were present in other varieties. Highly resistant types could be isolated immediately and no evidence was secured which indicated that resistance is developed as a result of constant association of the host plant and the pathogene. Resistant strains do not lose their resistance on wilt-free soil. Different degrees of resistance which were dependent on genotypic differences were observed. Although resistance is relative, the character is definitely inherited and probably multiple factors are involved.

Resistance to Black Stem Rust and Smut in Oats

Several physiologic strains of black stem rust of oats, *Puccinia graminis Avenae* E. & H. have been discovered. White Tartar (White Russian) is resistant to strains found in this country (Stakman, et al., '23). In the studies of resistance to black stem rust in Minnesota the first series of hybrids consisted of crosses of White Russian, *Avena sativa orientalis*, and Minota and Victory, *A. sativa*, two desirable mid-season oats which are susceptible to stem rust. Garber ('22) found resistance to be a dominant character to susceptibility and ratios approximating 3 resistant to 1 susceptible were obtained in F_2. The inheritance of resistance and susceptibility appeared independent from the inheritance of open and side panicle. By studying the reaction of small F_3 families in the seedling state in the greenhouse (Griffee '22) it was possible to isolate rapidly large numbers of F_3 lines that were homozygous for resistance to black stem rust. Garber obtained some indication that the side-panicled segregates yielded less on the average than the open-panicled types, therefore, open-panicled segregates were selected.

White Russian is late in maturity compared with Victory and Minota. From the Minota-White Russian cross there were obtained several lines which matured nearly as early as 60-day oats.

It has been found in Minnesota that a large proportion of the lines, if selection each year is made on the individual plant basis, breed approximately true in F_5. In recent years the selection of homozygous types from crosses has been deferred until F_5. Over 250 lines which were homozygous for rust resistance and apparently otherwise desirable have been tried out in rod row tests. Several of the better ones have been tried also in 1/40th acre plot trials. Since nearly all lines yielded well it has been very difficult to discard on a yield basis in the rod row trials by the probable error method. Yields in 1/40th acre plots of several promising selections are given in Table 1 in comparison with Minota and Victory.

It will be noted that the hybrids are the equal of the standard varieties Minota and Victory and it seems that Minn. No. 686 is superior in yielding ability. There was considerable rust at Waseca and Crookston. Averages for the percentage of rust infection are given in Table 2.

TABLE 1. YIELD IN BUSHELS PER ACRE OF HYBRIDS RESISTANT TO BLACK STEM RUST AND OF MINOTA AND VICTORY. PLATS ARE 1/40th ACRE. AVERAGE 1924 AND 1925.[2]

Variety and No.	Univ. Farm	Waseca	Morris	Crookston	Average
Minota, 512	60.8	86.4	79.3	76.8	75.8
Victory, Acc. 514	59.6	80.5	79.8	72.7	73.2
Wh. Russian×Victory, 686	60.6	95.7	85.1	80.3	80.4
Wh. Russian×Minota, 687	66.2	75.3	73.8	84.3	74.9
Wh. Russian×Minota, 690	68.7	79.0	73.8	77.4	74.7

TABLE 2. PERCENTAGE OF STEM RUST OF MINOTA, VICTORY, AND RUST RESISTANT HYBRIDS. AVERAGE 1924 AND 1925, 1/40th ACRE PLOTS

Variety and No.	Waseca	Crookston	Average
Minota, 512	31	54	42.5
Victory, Acc. 514	26	66	46.0
Wh. Russian×Victory, 686	0	7	3.5
Wh. Russian×Minota, 687	0	1	0.5
Wh. Russian×Minota, 690	0	2	1.0

Two of the rust resistant hybrids were grown in the uniform rust nurseries of the Office of Cereal Crops and Diseases, Bureau of Plant Industry[3]. There were 41 stations in all comprising tests in the following states:

Great Plains Region. Dakotas, Kansas, Nebraska, Wyoming, Colorado, Oklahoma and Montana.

Great Lakes Region. Wisconsin, Michigan, Indiana, Ohio.

Mississippi Valley. Minnesota, Iowa, Missouri, Illinois, Kentucky, Tennessee.

The rust resistant hybrids proved rather highly resistant in all tests.

Several years ago crosses were made between several desirable homozygous rust resistant lines of the White Russian crosses and Black Mesdag which, according to Reed ('20), is immune for smut, *Ustilago avenae* and *U. levis*.

Black Mesdag also differs from other parental strains in color of hull and development of awns. It was found that color segregated in a ratio of 3:1 in this cross and that resistance and susceptibility to rust were dependent upon a single factor pair as in the original crosses. There appeared to be no close linkage between rust reaction and the other differential characters (Lunden '24).

Reaction to smut was studied by growing F_3 and F_4 lines under field conditions. Before being sown the 5 grams of seed used for each 10-foot row were mixed with about a teaspoonful of smut. The degree of smut infection was not very great although there were some smutted plants in each row of the smut-susceptible parents and no plants of Black Mesdag were infected. By harvesting the F_3 lines in bulk, except for the selection of several desirable panicles from some of the more promising lines, and by growing two F_4 rows from each F_3 line that produced no smut, a fairly accurate estimation of reaction has been

[2] Data were furnished by A. C. Arny who is in charge of variety tests.

[3] These nurseries are under the direction of Doctors E. C. Stakman and M. N. Levine, who kindly furnished a statement of the results.

obtained. There was good agreement in F_4 for the replicates for the various strains. Some lines produced no smut in F_3 and only slight infection in F_4. These have been classed as resistant. Those classed as immune have been smut-free in F_3 and F_4. Similar numbers of resistant and susceptible, black and white, heavily awned and weak awned F_2 plants were selected at random and their progeny studied in later generations in their reaction to smut.

The data for smut reaction, rust reaction, and color of hulls are given in Tables 3 and 4.

TABLE 3. REACTION TO RUST IN F_2 AS DETERMINED BY F_3 BREEDING BEHAVIOR IN RELATION TO REACTION TO SMUT AS DETERMINED BY BREEDING BEHAVIOR IN F_3 AND F_4 (S=SUSCEPTIBLE; R=RESISTANT; I=IMMUNE)

	Susceptible to rust			Resistant to rust		
Reaction to Smut	S	R	I	S	R	I
Numbers	159	10	24	130	12	36

TABLE 4. COLOR OF GLUME IN F_2 AS DETERMINED BY F_3 BREEDING BEHAVIOR IN RELATION TO REACTION TO SMUT (S=SUSCEPTIBLE; R=RESISTANT; I=IMMUNE)

	Black glumes			White glumes		
Reaction to smut	S	R	I	S	R	I
Numbers	145	12	16	145	11	44

The parents in these crosses were of the following characteristics: (1) Black Mesdag—black glume, heavy awn, immune to smut, susceptible to rust; (2) White Russian—hybrids, white glumes, weak awn, susceptible to smut, resistant to rust.

It will be observed that a greater percentage of the resistant than of the susceptible genotypes are immune to smut and that a greater proportion of the white glumed plants are immune to smut than of the black. This is opposite to the relationship in the parents.

Weak and strong awns segregated in F_2 but the results were complex. There are now available many weak awned lines that appear resistant to rust and immune from smut. Some of the hybrids look very promising and there seems to be every reason to expect that desirable agronomic types resistant to black stem rust and immune from smut may be obtained from these crosses.

Black Stem Rust of Wheat

Black stem rust of wheat caused by *Puccinia graminis Tritici* E. &. H. often causes enormous losses to the wheat grower in the spring wheat belt and is a rather frequent cause of a partial crop failure. A consistent effort to obtain resistant varieties which are desirable otherwise has been made for over fifteen years. Rather extensive studies of rust resistance are being made by the state experiment stations of Minnesota, Wisconsin, and North Dakota and by the federal Department of Agriculture, and recently the Canadian Government has undertaken the problem of breeding rust resistant wheats.

Early in the studies it was observed that in some cases a wheat that was resistant one year might prove susceptible in some other season. The discovery of physiologic races of rust that could be differentiated only on the basis of their manner of reaction on wheat varieties furnished a logical explanation of the variable reaction of varieties in different seasons. Thirty-seven physiologic races of black stem rust of wheat have been described by their manner of reaction on twelve varieties of wheats belonging to the species *Triticum vulgare*, *T. compactum*, *T. dicoccum*, *T. durum*, and *T. monococcum* (Stakman and Levine '22) and several other physiologic races are known. The problem of breeding wheats resistant to all forms seems a difficult one, although perhaps not as difficult as it would seem on the basis of the number of physiologic races of rust. As has been mentioned, it appears that physiologic forms of rust are as constant as other plants and that "rust resistance is comparable with other permanent characters" (Stakman, et al., '18, '18[a]).

As some varieties of *T. durum* have been highly resistant for many years there seems every reason to believe that permanent resistance to black stem rust may be obtained. The present discussion will refer mainly to the following points: (1) Can the resistance of durum wheats be transferred to varieties of *T. vulgare?* (2) What is the genetic nature of resistance and how is the character inherited? (3) What is the present status of the problem of obtaining resistant varieties?

Crosses Between the 21 and 14 Chromosome Groups

Varieties of *T. durum* contain 14 chromosome pairs while varieties of *T. vulgare* contain 21 pairs. Great progress has been made by various investigators in cytological studies of wheat species hybrids. Fourteen chromosomes of *T. vulgare* are homologues of the fourteen haploid durum chromosomes, and in generations later than F_2 of durum-vulgare crosses there is a rapid return to the 14 and 21 chromosome condition, with the probability that gametes of intermediate number frequently are eliminated (Kihara '19, '21, '24; Sax '18, '21, '22; Watkins '24). Sax ('23) doubts the possibility that rust resistance can be transferred from varieties of *T. durum* to *T. vulgare* by crossing and apparently favors the hypothesis that rust resistance is frequently dependent in species hybrids upon the "physiologic condition of the host." In studies conducted by Sax accurate correlated data of various morphological characters, chromosome numbers, and rust resistance were taken. The number of plants worked with was small and not extensive enough to warrant a conclusion as to the possibilities of transferring characters from durum to common wheats. Moreover, other characters such as grain color can be transferred from 14 chromosome wheats to the 21 chromosome group and rust resistance and susceptibility are conditioned by genetic factors. Thompson ('25), in a similar study, found that resistance to rust could be transferred from durum to common wheats although the common wheat segregates were not as resistant as the durum parents. Thompson found that the vulgare-like segregates commonly showed one or a few durum characters.

Rather extensive studies have been conducted in Minnesota (Hayes, et al., '21, '20) in attempts to transfer the rust resistance from durums to common wheats. From crosses of Marquis × Iumillo a homozygous selection bearing the nursery number II–15–44 has been obtained and named Marquillo. This new variety is rather rust resistant although in this respect not the equal of the Iumillo parent. Futhermore, it appears from crosses of this new variety with common susceptible wheats that the rust resistance of Marquillo is dependent upon at least two main genetic factors. These factors have been transferred from Iumillo to a vulgare segregate with 21 pairs of chromosomes. This new variety appears about the equal of Marquis in milling and baking qualities although it is rather susceptible to root rot, a character obtained without doubt from its durum parent (Hayes, et al., '25).

In a cross between Mindum, *T. durum*, and Velvet Chaff, *T. vulgare*, a homozygous segregate which appeared to be a vulgare wheat, was obtained which was highly resistant in the seedling state in the greenhouse to two physiologic forms of rust to which Mindum was resistant and vulgare wheats commonly are susceptible.

McFadden ('25), in South Dakota, has studied large numbers of plants of a cross of a resistant emmer, *T. dicoccum*, with Marquis. Common wheats have been obtained which have proved highly resistant for many years in his plots. One of these was grown in Minnesota in 1925 and proved very highly resistant. From counts made by Griffee it is known to belong to the 21 chromosome group.

These results prove conclusively that rust resistance can be transferred from durum and dicoccum wheats to the 21 chromosome group.

Some genetic studies of rust resistance. As there are many physiologic races of rust which can be differentiated only on the basis of their reaction on particular wheat varieties it seems reasonable to expect numerous genetic factors which condition resistance and susceptibility. In some cases resistance and immunity are dominant to susceptibility and in other crosses susceptibility has been found to be dominant to resistance.

Several crosses have been carefully studied where the parents reacted in a reciprocal manner to two physiologic races of rust. These comprise crosses of durum × vulgare, durum × durum, and vulgare × vulgare. In each case varieties were obtained which contained factors for resistance or immunity to both rust forms and other varieties which were susceptible to both (Puttick '21; Harrington and Aamodt '23; Hayes and Aamodt '23).

There are two sorts of resistant varieties. Some varieties are highly resistant in the greenhouse in the seedling state, and these varieties, when mature, are resistant to the same physiological forms of rust under field conditions. Some varieties, however, are rather susceptible in the seedling state in the greenhouse to certain physiological forms but they may be highly resistant to these same forms under field conditions when approaching maturity. The resistance in the seedling state is believed to be physiological in nature while there is some evidence that the resistance of plants when approaching maturity is dependent upon morphological characters (Hursh '24).

Kanred, a winter wheat variety of the Crimean group, is immune from 11 physiologic forms of rust and highly resistant to 9 others. The immunity of Kanred is a dominant character in crosses and dependent upon a single factor pair (Aamodt '23). If resistance to 20 physiological races is controlled by a single genetic factor the problem is simplified greatly (Hayes and Stakman '22).

There is some evidence that the field resistance to many and perhaps all physiologic forms of Marquillo (the 21 chromosome segregate from the cross of Iumillo and Marquis) is dependent upon 2 main pairs of factors and that these are independent in inheritance of the factor for immunity carried by Kanred. Moreover the resistance of Marquillo and of Kota (the rust resistant variety of *T. vulgare* produced from a plant selection at the North Dakota station) appears to be dependent upon different factors since F_2 crosses of Kota × Marquillo are preponderantly susceptible, although resistant types can be recovered.

The present status of the problem. Large numbers of physiologic races of rust have been discovered. Resistance to many physiologic forms may be conditioned, however, by a single genetic factor. The resistance of two parent varieties of wheat which react reciprocally to two physiologic forms of rust can be combined in a single variety.

Many vulgare wheats are now known which are highly resistant to one or many physiologic forms. Kota, Marquillo, and Kanred have already been mentioned. Ceres, obtained at North Dakota from a cross of Kota × Marquis, has good strength of straw, a character which Kota lacks, and appears nearly as resistant as Kota although not quite the equal in rust resistance of Marquillo.

Several vulgare wheats are available which are highly rust resistant, both in the seedling state and under field conditions to certain physiological forms. Notable examples are rust resistant segregates of crosses between Emmer and Marquis obtained by McFadden in South Dakota; Webster, a selection which Stakman and others ('25) found resistant to many physiologic forms in the greenhouse; and a selection from a cross of Sevier × Dicklow described by Stewart ('26) which has proved resistant both in the seedling state and in the field. Obtaining a variety resistant to all physiologic forms seems only a matter of time.

Resistance of Barley to Helminthosporium Sativum

In the previous studies of disease resistance reported here the problem of controlling the disease was the central feature of the study. Until a rather recent period it has not been known that resistance in barley to *Helminthosporium sativum* was of importance in Minnesota. The reason is that the standard varieties of barley grown in this section are rather highly resistant to this disease. The importance of resistance to *H. sativum* became apparent in a study of smooth-awned hybrids which were obtained from crosses of Lion, a black, 6-rowed, smooth-awned variety with Manchuria, a white, 6-rowed, rough-awned variety. Some of the purified hybrids yielded well in preliminary trials but in later trials they were found to be susceptible to the "spot blotch" disease by *H. sativum* (Harlan and Hayes '19; Hayes and Stakman '21).

Studies of varietal resistance and of inheritance of reaction to *H. sativum* have been made. The barley varieties and hybrids studied have been grown in infested soil and sprayed at about heading time, at intervals of a day or two, with spore suspensions of *H. sativum*. The following description of methods is quoted from a previous paper (Hayes, et al., '23):

> The spraying was done between 7 and 8 p.m., from three to five sprayings being made.
>
> Data on the degree of infection were taken when the barley was nearly mature. Separate notes were taken on the degree of infection of heads, culms and nodes, and roots. The terms "heavy," "medium," "light," and "trace," were used to denote degrees of infection; and plus and minus signs further to differentiate the severity of the infection. For more exact comparison, the various degrees of infection were denoted by numerical figures as follows:

trace =10	medium− =6	heavy − =3
light − = 9	medium =5	heavy =2
light = 8	medium+ =4	heavy + =1
light + = 7		

> These figures allowed a summary of the infection of the separate notes taken on 3 different parts of the plants, that is, spike, culm, and leaves together, and root, and also aided in determining the degree of correlation between reactions obtained in successive seasons or between the degree of infection of different parts of the plants of the same varieties.

It will be noted that 30 represents the highest possible degree of resistance and that 3 represents complete susceptibility.

In 2 cases there was an opportunity to correlate yielding ability in rod row trials with degree of infection in the specially prepared disease garden. The correlation for yield and disease reaction was 0.445 ± 0.087 for 39 smooth-awned hybrids for the years 1921–22 which proves that resistance to this disease is of considerable economic importance.

In the first studies of inheritance a cross between Lion and Manchuria was used. Lion is susceptible to *H. sativum* while Manchuria is resistant. Resistance and susceptibility are not absolute, however, and are greatly influenced by environmental conditions. One hundred and twenty-four F_3 lines were grown in the disease nursery. Of this number, 8 lines were as susceptible in F_3 and F_4 as the Lion parent and 6 lines were as resistant as Manchuria. A greater proportion of resistant than of susceptible lines was of white color and characterized by rough awns, although within the 124 lines there were all combinations of other differential characters and of resistance and susceptibility. This suggested a genetic linkage. One of the difficulties of accurate analysis was variability of reaction in different parts of the field. This difficulty was obviated partially in later studies which were made by Griffee ('25), by growing 2 replicated rows of each F_3 hybrid studied. In the cross studied by Griffee the following parents were used:

Svanhals, 2-rowed, white glume, rough awn, resistant to *H. sativum*.

Lion, 6-rowed, black glume, smooth awn, susceptible to *H. sativum*.

The character pairs 2-rowed vs. 6-rowed, black vs. white glumes, rough vs. smooth awns, were found to be independently inherited.

Resistance and susceptibility tend to be correlated with each of these 3 character pairs. That the correlation is genetic may be inferred, as breaks in

the linkage relation have been found and all combinations of resistance vs. susceptibility, black vs. white, 2-rowed vs. 6-rowed, and smooth vs. rough awns have been obtained. This leads to the conclusion that 3 genetic factors or groups of factors are concerned with manner of reaction to *H. sativum* in the Svanhals-Lion cross.

Three varieties have been obtained from a cross of Manchuria with a smooth-awned segregate of the original Lion × Manchuria cross. These new varieties have been named Velvet, Comfort, and Glabron. They have been extensively tested in replicated rod row trials at University Farm, St. Paul, and have been grown for 3 years in replicated 1/40th acre plots at University Farm, Waseca, Crookston, Morris, and Grand Rapids. All 3 varieties appear the equal in yielding ability of Manchuria, Minn. 184, which is the standard 6-rowed variety recommended in Minnesota. Glabron appears much superior to Manchuria, Minn. 184, in strength of straw.

Summary

The importance of obtaining disease resistant varieties of crop plants is very great and the success or failure of a crop is dependent often upon its reaction to disease.

In the breeding of disease resistant varieties it is as important to study variability in the pathogene as in the host plant. Many plant parasites have been found to consist of physiologic races which can be differentiated only by their manner of reaction on different varieties. Because of the nature of the problem there is an opportunity for cooperative study with the geneticist and pathologist primarily responsible for its solution.

Resistance to disease is often not absolute and the extent of infection is greatly influenced by environmental conditions. It is important, therefore, with disease resistance as with other characters to study the effects of conditions upon the character which is being studied. Some method of inducing an epidemic must be used. Replication has been found to be a desirable means of obtaining reliable data.

A knowledge of the genetic factors involved in reaction to diseases aids materially in producing disease resistant varieties. Resistance and susceptibility are inherited in the same manner as other characters. After learning the nature of the parasite, the normal reaction between parasite and host plant, the environmental factors which condition reaction to disease, and some means of producing an artificial epidemic of the disease in question, the breeding of disease resistant varieties can be carried on in much the same manner as the breeding for other important characters.

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BREEDING WHEAT FOR DISEASE RESISTANCE[1]

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The leaf rust of wheat, *Puccinia triticina* Erikss., is one of the most widespread diseases of the wheat crop in the United States. Although found more or less abundantly in most years in practically every section of the country, it is most prevalent and is most harmful where humid conditions prevail. As the soft winter wheats are grown almost exclusively where humid conditions normally prevail, these wheats usually are most severely infected. In seasons of high rainfall in the semi-arid areas the hard winter and hard spring wheats may also suffer severe infection. In many years, therefore, leaf rust is a factor of considerable importance in the production of wheat.

Cooperative investigations on the inheritance of leaf rust of wheat have been under way for several years, by E. B. Mains and the writer, and later by C. O. Johnston. (Mains, E. B., Leighty, C. E., and Johnston, C. O. Inheritance of resistance to leaf rust. *Puccinia triticina* Erikss., in crosses of common wheat, *Triticum vulgare* Vill. Jour. Agr. Research **32:** 931–972. *Illus.* 1926.) Mains and Jackson have investigated the forms of leaf rust and have found that there are at least 12 distinct physiological forms. (Mains, E. B. and Jackson, H. S. Physiological specialization of the leaf rust of wheat, *Puccinia triticina*. Phytopathology **16:** 89–120. 1926.) Wheat varieties differ in their reaction to these several forms. Certain varieties are resistant to one or more forms and susceptible to others. Other varieties are susceptible to one or more of the forms to which others are resistant, and resistant to forms to which others are susceptible. No variety has been found, however, which is highly resistant under all conditions to all physiological forms of the rust.

From observations made on varieties in the field it was apparent early in the investigations that no one variety combined the resistance desired and the other qualities necessary in a wheat of commercial importance. The need for varieties resistant to rust appeared most urgent in the humid areas where the soft winter wheats are grown. No variety of adapted soft red winter or soft white winter wheat was found which was resistant to all the forms of rust commonly found in the humid areas. It was observed, however, that certain hard red wheats, of the type commonly grown in the semi-arid areas, were more or less resistant to the physiological forms occurring in the humid areas. But these hard red wheats cannot be grown profitably in the humid areas. Despite their re-

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sistance to rust they do not yield as well as the adapted, though susceptible soft wheats.

The possibility thus presented of crossing these resistant, unadapted, hard wheats with the susceptible, adapted, soft wheats, and the development thereby of resistant, adapted, soft wheats, was utilized. In 1920 a series of hybrids was produced at Arlington Experiment Farm, near Washington, D. C., between several strains of Kanred, a hard red wheat developed in Kansas, and several other varieties of soft red, soft white, and other classes of common wheat. In 1921 a similar series of hybrids was produced in which Malakoff, another variety of hard wheat, was used as the resistant parent.

The early generations of these hybrids have been grown either in the greenhouse or field at Arlington Farm, the Tennessee Agricultural Experiment Station, and the Indiana Agricultural Experiment Station. Later generations have been grown at several other places. The object has been to determine the reaction of the segregates toward leaf rust in an effort to develop strains resistant to rust, while possessing other characteristics necessary in a commercial wheat.

Studies of the inheritance of resistance have been made both in the field, under natural conditions of infection, and in the greenhouse, where artificial inoculations under controlled conditions have been made. Field studies are complicated by several factors. No control can be exercised over the physiological forms infecting the plants. Several forms usually are present at any one place. Climatic conditions may favor or retard development of the rust and of the plants. Early plants may escape a late-appearing infection and may therefore be incorrectly classified as resistant. The different adaptations of the parents to growing conditions in any locality may influence the reaction of segregates, as it appears that rust development is proportional to the vigor of the host. Despite these and perhaps other conditions unfavorable to the determination of reaction toward rust in the field, it was evident that segregation for rust resistance occurred. Strains have been isolated that have maintained their resistance through several years when grown at any one place. Some of these appear to have other characteristics necessary to make them of practical value. It appears from tests to date that in some cases adaptation to a locality has been combined with resistance to the forms of rust prevalent in that locality. This cannot be said with certainty, for complete agronomic tests remain to be accomplished.

Accurate determination of the manner of inheritance of rust resistance has not been possible under field conditions. Fortunately, however, accurate determinations have been possible under the controlled conditions of the greenhouse. Results obtained on the F_2 and F_3 of hybrids between Malakoff and 9 other varieties of common wheat are shown in Table 1. Inoculations were made in the seedling stage, physiological form 12 of leaf rust being used on the F^2 and form 3 on the F_3. These 2 different forms were used because the culture of form 12 was lost in the time intervening between the growing of the F_2 and F_3, and form 3, which has the same reaction as form 12 on the parental varieties was substituted.

In F_2, segregation for resistance and susceptibility occurred in approximately a 3:1 ratio, with resistance dominant. The deviation from this ratio in all crosses is less than 3 times the probable error, with 1 minor exception which in F_3 also gave aberrant results. The average for all crosses is also very near a 3:1 ratio. The results in F_2 were confirmed by those obtained in F_3. In all crosses but one the ratio obtained was very close to 1 homozygous-resistant family: 2 heterozygous families: 1 homozygous susceptible family. Nearly all F_2 plants classed as susceptible produced only susceptible plants in F_3. Of the resistant F_2 plants approximately 1 out of 3 produced only resistant F_3 plants, while 2 out of 3 produced in each case approximately 1 susceptible to 3 resistant plants. It appears, therefore, that the resistance of Malakoff to the forms of leaf rust used is dominant in crosses with the 9 varieties used, and that resistance is due to a single mendelian factor.

Somewhat different results were obtained in a cross made at Manhattan, Kansas, between a strain of Fulcaster wheat, resistant to physiological form 9, and Kanred, which is susceptible to this form. F_1 plants were somewhat more resistant than Kanred in the seedling stage, but in the shooting and heading stages they were practically intermediate in respect to their parents in their reaction to form 9. In the F_2 the heterozygous plants became progressively more resistant from the seedling to the heading stage. Homozygous-resistant and homozygous-susceptible plants, however, maintained their reaction throughout the different stages of growth. These results, confirmed in the F_3, indicate that, in the cross between Kanred and this resistant strain of Fulcaster, resistance is recessive and is due to a single main-factor difference, with possibly modifying factors which cause changes in the reaction as the plants grow older.

As I have stated, no variety of wheat has been found that is resistant to all of the known physiological forms of leaf rust. Malakoff is resistant to 6 of the 12 known forms and susceptible to 6. A strain known as C. I. 3778 also is resistant to 6 forms, 3 of these being forms to which Malakoff is susceptible. Another strain recently named Webster (C. I. 3780) is resistant to 8 forms, the 6 to which Malakoff is resistant and 2 additional forms. Of the various strains of wheat some are resistant to certain physiological forms of leaf rust and susceptible to others. To each known physiological form some strain of wheat is resistant.

This being the case the problem presents itself of combining in one individual the resistance of the several strains of wheat to the several physiological forms of rust. This is necessary to effect general control of the disease, for several forms usually are present in any locality, and the forms are variously distributed. Changes also may occur from time to time in any given place.

In order to test the possibilities of combining the resistances possessed by different strains of wheat to different physiological forms of rust, crosses were made between several varieties differentiating these forms. The results may be illustrated by the cross between Malakoff and C. I. 3778.

F_2 seedlings of this cross were inoculated in the greenhouse with a culture of form 12 of leaf rust. Malakoff is highly resistant to this form and C. I. 3778 is highly susceptible. As shown in Table 2, of the 85 plants tested 65 were resistant

and 20 highly susceptible. This is a very close approximation to a 3:1 ratio, with resistance dominant. After these data on the reaction to form 12 were recorded, the infected leaves were trimmed off and the same 85 plants were inoculated with form 5. To this form Malakoff is susceptible and C. I. 3778 resistant, their reaction being the reverse of that to form 12. The reaction of the 85 plants to form 5 also is shown in Table 2. It is almost exactly the reverse of that to form 12, only 21 plants being resistant, while 64 were susceptible. This is almost exactly a 3:1 ratio with susceptibility dominant.

When the reaction of each of the 85 plants to both forms of rust is considered it is found, however, that their reaction has not been exactly reversed. The plants are grouped in 4 classes as shown in Table 3. The numbers in these 4 groups are very close to a 9:3:3:1 ratio, χ^2 being 0.083, indicating a very close fit. The resistance of Malakoff to form 12, and of C. I. 3778 to form 5, therefore, appears to be each dependent on a single independently inherited factor.

TABLE 3. NUMBER OF F_2 PLANTS OF THE WHEAT HYBRID MALAKOFF × C.I. 3778 SHOWING COMBINATION OF SUSCEPTIBILITY AND RESISTANCE TO PHYSIOLOGICAL FORMS 5 AND 12 OF LEAF RUST[1]

R. to F. 5 R. to F. 12	R. to F. 5 S. to F. 12	S. to F. 5 R. to F. 12	S. to F. 5 S. to F. 12	Expected segregation on 9:3:3:1 basis	χ^2	P
16	5	49	15	16:5:48:16	0.083	Very close fit

It should be noted that 16 F_2 plants of this hybrid combination were resistant to both forms of rust, the resistance of the 2 parents being combined in them.

Similar results were obtained in 2 other hybrids between varieties differentiating different forms of leaf rust. In every case the resistances possessed by the different varieties are inherited independently, thus tending to confirm the conclusion that resistance in each case is due to an independently inherited factor. It is interesting here to note that when the parent Malakoff is resistant, dominance of resistance occurs, but when Malakoff is susceptible dominance of susceptibility occurs.

Only 56 of the 85 F_2 plants of the cross between Malakoff and C. I. 3778 produced enough seed for F_3 tests. The plants of these 56 F_3 families were again inoculated in the seedling state first with form 5 and later, after infected leaves had been trimmed off, with form 11 (form 12 not being available, form 11, which behaves the same as form 12 on Malakoff and C. I. 3778, was used). Without going into details it may be said that the reactions of F_3 completely confirmed the F_2 results. Most important is the behavior of the 16 F_2 plants resistant to both forms of rust. Of these 16 there were 15 tested in F_3. All of these were resistant when inoculated with form 5. This is according to expectation as it is recalled that resistance was recessive in F_2 inoculations with form 5. When inoculated with form 11, to which the reaction in F_2 showed resistance dominant, 8 families were heterozygous, segregation occurring in the ratio of approximately

[1] R = Resistant; S = Susceptible; P = Physiological form.

TABLE 2. REACTION OF THE F_2 PROGENY OF THE HYBRID MALA

Parent or Hybrid Combination	Physiological forms	Number of plan			
		Class 0	Class 1	Class 2	(
Malakoff, C.I. 4898 ×C.I. 3778	5	12	8	1	
	12	17	30	18	
Malakoff, C.I. 4898	5	..	..	..	
	12	22	..	..	
C.I. 3778	5	..	15	..	
	12	..	..	..	

.KOFF × C.I. 3778 TO PHYSIOLOGICAL FORMS 5 AND 12 OF LEAF RUST

ts in reaction classes				Segregation per 4 plants	Deviation	$\frac{\text{Dev.}}{\text{P. E.}}$
Class 3	Class 4	Total resistant	Total susceptible			
..	64	21	64	0.99:3.01	0.01±0.13	0.08
..	20	65	20	3.06:0.94	.06± .13	.46
..	25	..	25	....	....	....
..	..	22	..	....	....	....
..	..	15	..	....	....	....
..	18	..	18	....	....	....

1 susceptible to 3 resistant plants. Seven families involving 245 plants bred true for resistance to form 11 and were thus resistant to both forms of leaf rust. When tested in F_4, progenies from 5 (all of the families tested) of these 7 resistant F_3 families maintained their resistance to both forms 5 and 11 in every case. These strains therefore combine the resistance of both parents and are breeding true for this resistance. Similar results have been obtained in other crosses studied.

The combination of the resistance of the parents to different forms of leaf rust is similar to the results obtained by Puttick and by Harrington and Aamodt in their work with the stem rust of wheat (*Puccinia graminis* Pers.). These workers succeeded in combining the resistance of the 2 parents to different forms of this rust.

It appears, therefore, that there is hope of obtaining, by means of hybridization of resistant parents, strains of wheat resistant to all forms of leaf rust. Whether or not this event is realized depends upon the amenability of other forms of rust to breeding methods. From results so far obtained it seems probable that strains resistant to 9 of the 12 known forms of leaf rust have been obtained, for Malakoff is resistant to 6 forms and C. I. 3778 to 3 others, to which Malakoff is susceptible. There exists, of course, the possibility that other forms of leaf rust will be found which may be more virulent, and for which no resistant varieties can be found. Again it may be that the rust forms themselves will mutate, as has been shown to occur in other forms of fungi. If mutations should occur in respect to the ability of such forms to infect previously resistant varieties, the accomplishments of breeding operations would be nullified. It is not likely, however, that all such untoward events would be coincident, and there is hope that the plant breeder will be able to keep pace with their progress.

THE DEVELOPMENT OF DISEASE-RESISTANT STRAINS OF CORN[1]

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Dent corn (*Zea mays indentata*) is one of the leading cereal crops in America. Directly and indirectly it contributes heavily to the prosperity of over ten million rural people in the United States, and probably as many urban people. Anything seriously affecting this crop and its value is of great economic importance and soon becomes one of our national problems.

ROT DISEASES CAUSE MUCH LOSS TO NATION'S CORN CROP

Approximately 10 years ago the attention of the United States Department of Agriculture and several of the State Agricultural Experiment Stations was directed to a group of diseases known as the corn root-, stalk-, and ear-rots. Perhaps it is a safe estimate that these diseases take a toll of from 10 to 15 per cent of the values of the nation's corn crop annually.

The rot diseases may cause a rotting of the seeds, a seedling blight, a rotting of the roots, reduced vigor, barrenness, nubbin-production, delayed maturity, and a rotting of the ears. Under favorable conditions ear-rots may spread and cause much added damage to the grain after maturity and before harvest, and also in storage. Thus the corn rot diseases are an important factor contributing to a lower yield per acre and a poorer quality of grain.

SELECTION AND BREEDING IMPORTANT CONTROL MEASURES

Losses from these diseases have been lessened by the following measures: soil management, seed selection, seed treatment, and the breeding of strains of corn resistant to disease and to injury from unfavorable conditions of soil and weather. It is not in the province of this paper to discuss soil management and soil treatment in connection with corn breeding, except to say that they are both important and deserve careful attention. Seedling blight resulting from seed infection with *Diplodia Zeae* (Schw.) Lev. and *Gibberella Saubinetii* (Mont.) Sacc. can be controlled to a large extent by several organic mercury compounds, comprising both dust and soak treatments. But the most effective methods of reducing losses from this group of diseases have been through seed selection and the breeding of strains resistant to disease.

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 19, 1926.

MUCH VARIATION IN DISEASE RESISTANCE OF OPEN-POLLINATED STRAINS

In connection with inoculation experiments conducted during the past 8 years, there has been much variation in the disease resistance and susceptibility of open-pollinated strains to injury following inoculation with *G. Saubinetii*, *D. Zeae*, and a species of *Pythium*. While some strains have been rather susceptible, other strains have shown considerable resistance. This degree of resistance in open-pollinated strains has been maintained over a period of years by careful selection.

Some commercial strains of corn widely grown in central Illinois have been found to be especially susceptible to ear-rot damage. Other commercial strains have been damaged to a much less extent under the same conditions. A few open-pollinated strains have been improved materially in disease resistance, particularly to ear-rots, during the last 5 to 10 years as a result of continued selection in that direction.

Thus the adoption of the better open-pollinated strains and their improvement by plant-, ear-, and germinator-selection has proved to be effective in reducing losses from these diseases and in raising the general quality of the grain.

RECOMBINATIONS OF SUPERIOR INBREDS MOST PROMISING METHOD OF CONTROL

However, the greatest contribution to the solution of the corn disease problems apparently must come through the development of highly disease-resistant, vigorous, and productive inbred strains, and their recombinations into first generation crosses, double crosses, and, perhaps, synthetic varieties that possess a maximum of good qualities and a minimum of undesirable qualities.

During the past 8 years we have conducted experiments to determine the reaction of a large number of inbred strains to pure-culture inoculations with several disease producing organisms. During that time inbreds have been isolated which have shown a wide range in resistance and susceptibility to each of the organisms (Plate I, upper view). Many strains may be highly resistant to 1 or 2 pathogens but susceptible to other pathogens. A few have proved to be relatively resistant to all the pathogens used, and a few have been found to be very susceptible to all. Some recombinations have proved to be highly resistant to comparatively all corn ills.

NEW RECOMBINATIONS MUST POSSESS MANY STRONG QUALITIES

Resistance to the rot diseases is of greatest value when it is combined with other good qualities. Many recombinations may be unusually high yielders but lack in strength of stalk to hold the grain erect until harvest. Others may be resistant to diseases affecting the crop before maturity but may be very susceptible to ear-rots. A few have consistently produced yields ranging from 130 to 147 bushels per acre with less than 1 per cent of rotted corn and no smut, but lacking in root anchorage. Others excel only on the very best soil. Any one of the above defects is sufficient to disqualify a strain of corn from being grown widely. If new recombinations are to be used extensively they must possess a

fine balancing of resistance to the major diseases with other necessary qualities. In many instances it may be necessary to sacrifice something in yield in order to increase quality and consistency of performance.

VIGOROUS INBREDS MAY BE OBTAINED

In 5 of the inbred families that have been carried 9 and 10 years there has been a marked improvement in both disease resistance and general vigor during the last 3 years. This must have been due, at least in part, to the elimination of the inferior material following plantings in cold soil and corn-sick soil, and by inoculation experiments. Such plantings make the more vigorous and the more disease-resistant plants sufficiently conspicuous to be selected and increased.

When inbreds, good from the standpoint of recombination, can be obtained that will produce from 60 to 75 per cent as much as the best open-pollinated corn, F_1 seed probably can be produced economically and sold at a figure that will make corn growers anxious to use it.

A first generation cross between this and another vigorous inbred yielded approximately 20 per cent more sound corn than the parent variety, matured about 10 days earlier, stood up well, and was highly resistant to ear-rots.

INBREDS AND RECOMBINATIONS VARY GREATLY IN FUNCTIONAL RANGE

Inbreds, apparently alike in all visible plant characters when growing under a favorable environment, may react very differently to unfavorable conditions of soil and weather, as well as to various disease-producing organisms. While a few will germinate and grow under a wide range of temperatures, others grow best only within comparatively narrow limits. Judging from the results secured from experiments conducted in control-temperature cases, some strains have a low temperature optimum, some an intermediate optimum, still others a high-temperature optimum. Resistance to and recovery from frost injury in the spring are closely associated with cold resistance. During the spring of 1925, a few inbred strains in our experimental plots near Bloomington were highly resistant to injury from a temperature of 24°F., when the plants were from 4 to 6 inches above the ground. The plants of other inbred strains were frosted down to the ground, but recovered. Other strains, with an equally good field stand, were almost completely killed under similar conditions (Plate I, lower view).

It has been found that root systems of inbreds and their recombinations differ significantly in both character and extent. While some corn root systems are very fibrous and extensive, others have few fibrous roots. Strains with few fibrous roots frequently have been found to be very susceptible to injury from drought. Fibrous root systems may differ greatly in their efficiency on account of their variability in resistance to root-rot. Two strains may have the same number of main and fibrous roots. In the one 95 per cent of the roots may be functioning, and in the other only 25 per cent.

Again, the proportion of tops to roots varies greatly in inbred strains. While some inbred strains have such a high proportion of tops in comparison with the roots that the plants lodge badly even when they are only knee-high, other

strains apparently devote the most of their energy to root production, even to the detriment of grain yield. There seems to be a physiological balance in the proportion of tops to roots for the best production of grain of high quality.

The same variation may be observed in resistance to stalk breaking, plant food economy, and light economy. Some strains are able to complete their maturation processes during a period of cloudy, rainy weather, whereas other strains remain at a standstill.

REACTION OF INBREDS AND RECOMBINATIONS TO A WIDE RANGE OF ENVIRONMENTAL CONDITIONS NECESSARY TO ESTABLISH THEIR MERIT

The importance of determining the physiological range and disease reaction of the various inbred strains soon becomes evident when an attempt is made to expand experimental possibilities into commercial realities. The yield that any set of inbreds and their recombinations will produce under favorable conditions is not nearly as important to the corn grower as the dependence and safety with which the inbreds and the recombinations can be produced, and the reliance that can be placed on their performance under the average conditions of a period of years. Some recombinations may have a comparatively wide range of usefulness, others may be best adapted to a rather restricted area.

This ability to perform well under a wide range of soil and climatic conditions, such as are encountered from year to year in almost any section of the Corn Belt, is dependent primarily on the functional range of the strains and crosses involved, as well as their reaction to the important diseases. Any one of several functions, for example, inability to germinate and grow well at a low temperature, susceptibility to seedling blights, drought susceptibility, poor economy in using plant nutrients, susceptibility to ear-rots, low resistance to lodging and stalk breaking, may prove to be a limiting factor under certain conditions and in certain seasons.

This in itself means that the greatest and most rapid success will be met in selfing, selecting, and recombining varieties which are standardized commercially in the various regions rather than introducing entirely new synthetic varieties.

MODERN CORN BREEDING NEITHER A ONE-MAN NOR A ONE-SCIENCE STUDY

It has become increasingly evident during the last few years that the most satisfactory progress in the study of disease resistance and susceptibility in corn, and in the development of disease-resistant strains possessing the other necessary physiological qualities, can be achieved only by the active cooperation of many workers and the correlation of their results. To say that any 1 science alone—whether genetics, physiology, pathology, chemistry—will solve, by itself, the great problems of corn breeding would be like contending that 1 cog in a wheel is all important or that 1 leg of a 3-legged stool is more important than either of the others.

We feel that the effective cooperation that has developed between the Office of Cereal Crops and Diseases of the Bureau of Plant Industry, the University of Illinois, the University of Wisconsin, the Funk Bros. Seed Co., and other

agricultural agencies, is an outstanding example of the value of combined effort. Geneticists, pathologists, physiologists, chemists, soil technologists, entomologists, and agronomists all have had, and will have an opportunity to contribute in such work, from their respective fields.

The University of Illinois has furnished 2 "Crop Production Fields" and more recently has added certain "University Soil Fields." But in corn breeding operations large areas of particular kinds of soil also are required. Such areas have been furnished by Mr. Eugene D. Funk of the above mentioned Seed Company, and by other interested farmers and corn breeders. Moreover, Mr. Funk and others, commercially interested, have contributed much in giving a clearer understanding of the actual needs, both present and future, in a "Program of Corn Improvement."

For their administrative contributions, much credit is due Dr. W. L. Burlison, Head of the Department of Agronomy, University of Illinois, Dr. C. R. Ball, Senior Agronomist in charge of the Office of Cereal Crops and Diseases, and also Dr. A. G. Johnson, Pathologist in charge of Cereal Disease Investigations.

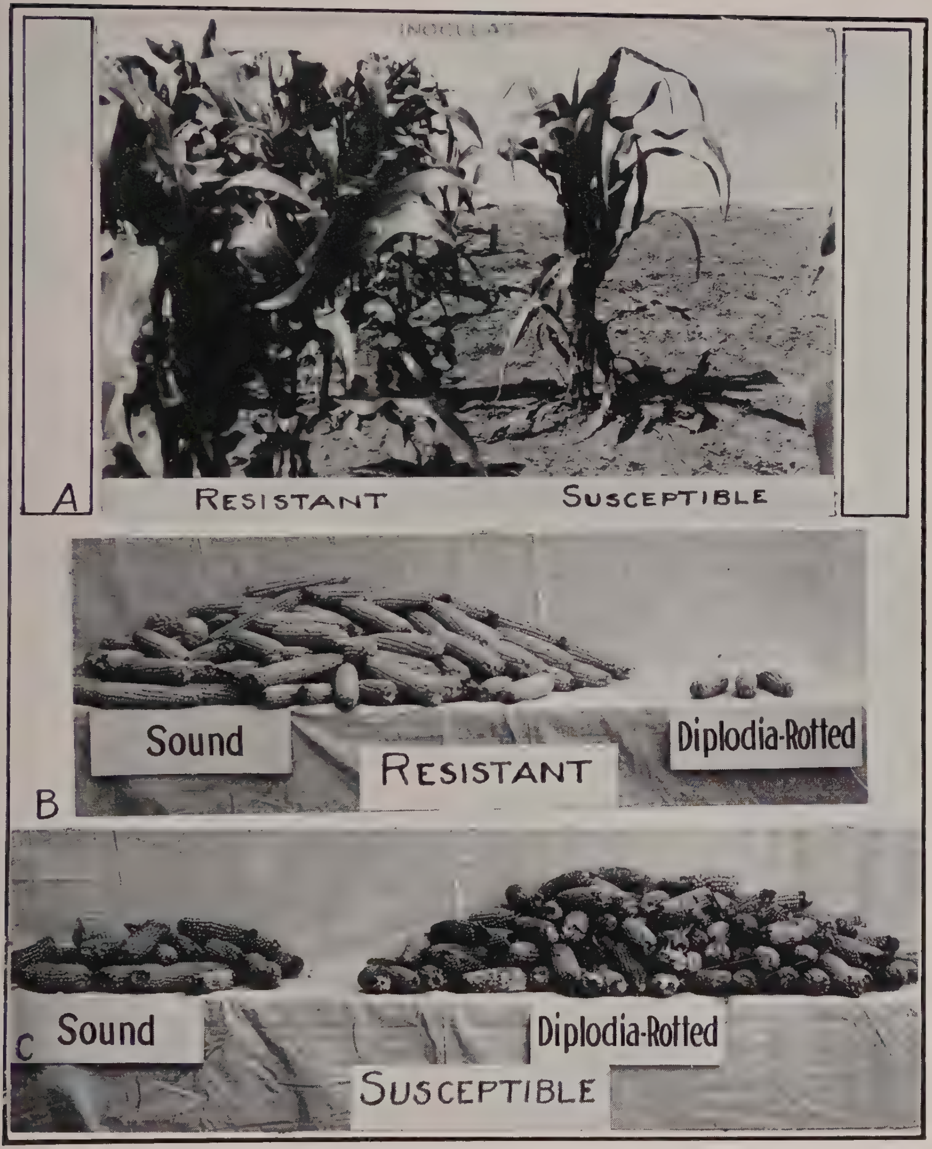
Explanation of Plates

PLATE I

Disease resistant, inbred lines of yellow dent corn. An inbred line resistant to *Gibberella* seedling blight (A, left) is shown in contrast with a susceptible line (A, right). Two similar inbred lines resistant and susceptible to *Diplodia* ear rot are compared in B and C.

PLATE II

Cold-Resistant and cold-susceptible inbred lines of yellow dent corn. Cold-resistant and susceptible inbred lines were planted in adjacent plots. The seedling stands were comparable when in the third leaf stage. On May 25 the temperature reached —4.4°C. without injury to the plants in Plot A, upper, whereas three fourths of the plants were killed in Plot B, lower. The photographs show the resulting stand at maturity.



HOLBERT AND DICKSON: DISEASE RESISTANT CORN

Holbert and Dickson: Disease Resistant Corn

L'INSTITUT DE BOTANIQUE APPLIQUÉE ET D'AMÉLIORATION DES PLANTES CULTIVÉES DE L'UNION DES RÉPUBLIQUES SOCIALISTES SOVIETIQUES[1]

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L'Institut de Botanique appliquée provient du Bureau de Botanique appliquée du Comité scientifique du Ministère d'Agriculture qui a été établi en 1894. Le but principal de ce Bureau est: (1) l'investigation des plantes cultivées de la Russie, de l'Europe et de l'Asie, et (2) les études sur les plantes nouvelles qui presentent l'intérêt pour l'introduction dans la culture.

Le premier directeur de ce établissement était M. le Dr. A. F. Bataline, connu par ses recherches sur les plantes oléifères, les blés sarrasins et d'autres plantes cultivées. Après Bataline c'était le Dr. A. A. Fischer von Waldheim, ensuite I. P. Borodine, membre délégué des sciences; depuis 1905 c'était le Dr. R. E. Regel qui était chargé de cette mission.

L'activité de cet Institut commence dès 1907. Par l'énergie de M. le Dr. Regel cet Institut devint déjà au commencement de notre siècle le centre de l'étude des plantes cultivées en Russie. Dès 1908 commence la publication du "Bulletin de Botanique Appliquée," qui à présent représente le journal central pour la botanique appliquée et l'amélioration des plantes en Russie. Jusqu'à présent 16 volumes du "Bulletin de Botanique Appliquée" et d'Amélioration des plantes cultivées ont été publiés.

L'immense territoire de l'Union des Républiques soviétistes socialistes, qui est presque plus d'une septième du monde entier, ne peut pas être une quantité négligeable dans l'économie rurale du monde et surtout dans les instituts des plantes cultivées. Les dimensions de ce pays ont obligés le Gouvernement de payer assez d'attention à l'Institut des plantes cultivées.

Pour faire la politique dans l'économie rurale, pour faire du progrès dans la culture des plantes il faut avoir pour ce territoire immense beaucoup d'information. Pendant les années des mauvaises récoltes, quand il faut importer les grains des autres régions, il est nécessaire de connaître la distribution géographique des variétés des plantes de culture. Il faut savoir exactement quelles variétés sont convenables pour telle ou telle région. C'est pourquoi même d'un point de vue pratique les études dans la botanique appliquée sont d'une importance dans ce vaste pays. Pendant les dernieres années de la guerre mondiale et au commencement de la révolution sociale notre Institut a éprouvé beaucoup de difficultés. Beaucoup de monde étant mobilisé pour la guerre, les ensemencements

[1] Presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 20, 1926.

furent suspendus. Avec la restauration du pays l'Institut a développé son travail et a trouvé même la possibilité d'augmenter et d'aprofondir son activité.

L'organisation de l'Institut. Exposons en quelques mots la structure de cet Institut.

L'Institut se compose d'abord des laboratoires généraux suivants: le laboratoire de Génétique (chefs: Dr. Vavilov et Karpetchenko), d'Amélioration des plantes (chefs: Dr. Pissarev et Dr. Govorov), de Physiologie appliquée (Dr. Maximov), de Physiologie et d'Anatomie (Dr. Levitsky), de Biochimie (Dr. Ivanov), de Technologie (Dr. Tchingo-Tchingas), un laboratoire, bien organisé pour les études des plantes textiles du point de vue de la technique et enfin un herbier général des plantes cultivées.

Le second groupe des institutions est dedié aux cultures différentes. Il y a des sections des plantes de hautes cultures (chef: Dr. Vavilov), la section pour l'étude des mauvaises herbes (chef: Dr. Malzev), des plantes potagères (Dr. Kitchunov et Boukassov), des plantes fruitières (Dr. Pachkevitch), des plantes subtropicales (Dr. Voronov et Markovitch).

Les sections sont divisées en *Departements*. Les cultures, étudiées en détail comme le blé, l'orge, l'avoine, le seigle, le millet, les plantes légumineuses, les plantes olifères, la pomme de terre, ont des chefs spéciaux. Pratiquement pour chacune de ces plantes il y a une ou deux chambres. Il a y un musée de collections. Chaque departement mène les études sur sa plantes. On travaille sur la géographie russe et étrangère. En général il y a à présent presque 30 departements.

Les collections des plantes sont assez vastes, par example pour le blé il y a plus de 15 mille spécimens—échantillons collectionnés dans toutes les régions de la Russie et des autres pays. Les collections pour les blés, le seigle, l'avoine, le millet et quelques autres plantes de la haute culture sont très complètes.

Ces departements font des études à moyen des semences, des études botaniques, par des expéditions spéciales, des études dans les différentes régions du pays. C'est comme un petit centres de tous les pays pour l'étude de toutes les cultures les plus importantes.

Chaque 3 ou 4 ans le departement doit faire des sémences de ses collections pour avoir toujours les spécimens en état vif. Ce travail est assez difficile et coute très cher. Pendant la guerre et pendant les premières années après la révolution ces ensemencements furent interrompus. C'était une grande perte, parce que beaucoup de collections ont perdu la capacité de germier.

Le troisième groupe des institutions se compose des *Stations expérimentales*, des champs expérimentals de l'Institut de Botanique appliquée, qui se trouvent à présent presque dans toutes les régions les plus différentes de la Russie. Quelques-unes de ces stations représentent des institutions bien organisées comme Svaleff; les autres sont les suivantes: dans le nord—(1) la Station Centrale à Detskoje Selo (Zarskoye Selo), près de Leningrad, avec ses divisions au Mourman ($67\frac{1}{2}°$) et dans de différentes régions du nord; (2) la Station de Moscou; (3) la Station des régions des steppes à Voronesh; (4) la Station au Caucase du nord de Kouban sur la terre végétale; (5) la Station de l'Ukraine,

la région des steppes; (6) la Station à Belarussie; (7) la Station d'Azerbaidjan près de Bacou; (8) la Station de Turkestan; (9) la Station subtropicales au bord de la Mer Noire à Suhum. Toutes ces stations sont plus ou moins grandes, mais nous avons encore des petits Champs d'Expérience.

On a établi les *Expériences géographiques* pour étudier les modifications des variétés des cultures dans les différentes conditions du vaste pays. Il y a déjà 4 ans que nous avons organisé les expériences géographiques. Cent quatre-vingt dix variétés différentes des plantes de la haute culture du printemps et d'automne sont sémées dans les différentes régions. L'année passée il y a avait 58 points; cette année-ci nous avons à peu près 90 stations. Ces expériences sont donc des informations sur la limite des cultures. Les résultats de ces essais sont analysés morphologiquement et par les analyses chimiques.

La Section des Essais de comparaison des variétés commerciales. Sous la direction du Dr. Talanov il existe depuis 3 ans une organisation très importante du point de vue pratique des comparaisons des variétés nouvelles, qui ont été reçues par la station d'amélioration des plantes et qui ont été importés de l'étranger.

Pour faire des expériences plus régulièrement et pour organiser la concurrence, on a organisé des essais d'étant. Les variétés définitives ont été semées dans les régions différentes par les stations expérimentales. Les semences aprés ces expériences ont été analysées dans les laboratoires de l'Institut de point de vue technique et chimique.

La Section de la Naturalisation des Arbres. Il y a déjà des années qu'on a organisé aussi une section de la Naturalisation des Arbres. Le vaste territoire de la région des steppes et des déserts, doit être semé artificiellement de forêts. Nos villes sont assez pauvres d'arbres, c'est pourquoi on a commencé de faire des études dans cette direction. A présent il y a quelques petites stations pour ces études. C'est surtout au sud de l'Union au Turkestan, à l'Ukraine, où doivent être déployées les travaux de la naturalisation des arbres. Cette section est liée aussi avec la station subtropicale parce que c'est surtout de la région subtropicale de notre pays où les travaux dans le domaine de la naturalisation doivent donner des résultats très démonstratifs.

La Bibliothèque et l'Administration. L'Institut a une bibliothèque spéciale sur la botanique appliquée. Il y a près de 40,000 noms (quarantes milles). Cette bibliothèque est assez riche en ouvrages botaniques, surtout sur les plantes cultivées. C'est la meilleure bibliothèque dans cette région de la Russie et c'est surtout la division des génétiques, qui est très complète.

L'Administration occupe un grand édifice à Leningrad, 44 Rue Herzen, ou se trouve aussi la rédaction du "Bulletin de Botanique Appliquée," ainsi que la bibliothèque populaire sur les plantes cultivées. Le personnel de l'Institut se compose de 125 personnes engagées dans le travail scientifique et de 200 téchniciens et ouvriers.

L'organisation des expéditions. Pour étudier le vaste pays, qui n'a pas assez de stations expérimentales jusqu'à présent et qui possède des régions très différentes, étant très riche en variétés, l'Institut est obligé d'organiser chaque

année quelques expéditions sur le territoire de l'Union. Ce sont surtout les régions de l'Arménie, du Caucase, du Turkestan, qui sont peu connues. Ces régions sont d'un intérêt exceptionel non seulement du point de vue patriotique, mais du point de vue général, parce que là se trouvent les régions de l'origine des plantes cultivées.

Les études scientifiques menées par l'Institut pendant les années dernières ont établi que le centre des origines des plantes cultivées se trouve dans les regions adjointes au Turkestan russe et le Caucase. C'est pourquoi l'Institut a organisé quelques expéditions en Perse, l'Afghanistan et la Mongolie pour faire des collections pour les travaux des stations expérimentales pour la correlation des plantes cultivées. C'est pourquoi l'Institut organisait chaque année quelques expéditions dans les pays différentes, ou se trouvent les centres des diversitées des variétés.

Les résultats des travaux scientifiques. Les résultats des ouvrages scientifiques sont publiés dans le "Bulletin de Botanique Appliquée." La tendance générale est la description des cultures du point de vue de la géographie botanique, et de la classification.

Le but fondamental de l'Institut est de préparer pendant les années prochaines des monographies plus ou moins complètes de toutes les plantes, qui sont d'importance pour la Russie et de donner la description de toutes les variétés, qui sont cultivées sur son vaste territoire. Pendant les études des variétés nombreuses des plantes différentes, collectionnées en toutes les régions des pays différents du monde, on a remarqué des régularités, qui se trouvent dans les variations des plantes, des espèces linnéans. L'étude systématique des variations a fourni des materiaux nécéssaires pour établir les schèmes des variations. A présent on a préparé des schèmes des variations sur les familles les plus importantes, connues, par example, les Gramineae, Papilionaceae, Cucurbitaceae, Cruciferae. Ces schèmes sont utiles pour étudier les plantes et pour les classifier, de même que pour les ouvrages pratiques en amélioration des plantes cultivées. Pour trouver les plantes absentes dans les schèmes et qui devaient exister en théorie. l'Institut a été obligé de faire des recherches dans les pays différents, d'étudier la distribution géographique des variétées des plantes. Ces recherches ont amené à des questions générales sur l'origine des plantes cultivées. Les résultats de ces ouvrages sont publiés dans une monographie, intitulée "Etudes sur l'origine des plantes cultivées." De tous les ouvrages publiés pendant les dernières années, nous mentionnons un vaste ouvrage du Dr. Maximov: "La base physiologique de la résistance des plantes contre la sécheresse;" une monographie critique sur cette question, qui est très importante pour une grande partie de la Russie, qui périodiquement souffre de la sécheresse. Cette monographie donne une revue critique de toutes les recherches de même que les resultats du laboratoire physiologique de l'Institut de Botanique. Parmi les ouvrages systématiques nous pouvons mentionner les monographies sur le blé, sur la pomme de terre, sur les plantes fouragères. Un vaste volume (vol. 13) et le volume 15 sont dédiés à la systématisation des blés. On a établi en Russie une espèce nouvelle, appelée *Triticum persicum*. Parmi les génétiques nous

pouvont mentionner les enlisses des orges, surtout des charactères des barbes lisses, des barbes dentées. Sur la génétique "Des espèces nouvelles du blé *Triticum persicum*," "Sur les croisements des espèces différentes des Cruciferae et sur les analyses des Cucurbitaceae."

Le laboratoire biochimique prépare à présent des rapports sur les analyses des plantes des essais géographiques. Toutes les variétés (190) des plantes différentes, qui sont cultivées dans les régions différentes, sont analysées. On a établi des résultats de 3 ans, des régularités, qui se trouvent dans les changements de la composition chimique des plantes. Les variations de la composition chimique de protéine sont très vastes dans notre pays. Il y a des régions, comme, par éxample, de sud-est de la Russie Européenne, Samara, Saratov, où le pour cent de protéine dans les blés du printemps va jusqu'à 25%. A présent on prépare des cartes, des modifications, des compositions chimiques du blé, d'orge, d'avoine et d'autres plantes. On a decouvert que les changements dans la composition chimique des plantes dans les montagnes sont assez analogues aux changements qui ont lieu dans la direction du sud au nord. Par éxemple, on a trouvé que la quantité des différents acides dans l'huile de lin est la même au nord que dans les hautes altitudes des montagnes. Nous avons trouvé de même que la hauteur du lin de la même ligne pure, du lin cultivé pour la fibre, est assez considérable dans les montagnes, même s'il est cultivé dans le sud.

Le principe général, qui domène dans toutes les branches des études, est le principe géographique. Pour comprendre les régularités qui gouvernent les phénomènes naturels sur ce vaste territoire, on doit regarder les choses du point de vue de géographie. Les modifications évoquées par le facteur géographique sont souvent plus fortes que celles des cultures des plantes de la nature antérieure.

La qualité technique et la qualité chimique, par éxemple, des céréales des fibres, dépend plus des conditions extérieures, des conditions du climat et de la terre, que de la variété. Pour organiser la culture des plantes il est nécessaire d'établir régulièrement par ces essais géographiques les régions optimo, pour les cultures, les régions ultimo pour la production du sucre, du protéine, de l'huile. Les exigences géographiques et les analyses chimiques ont établi que la qualité, le pour cent de l'huile ne change pas beaucoup dans les mêmes variétés de lin. Mais la qualité de l'huile est très différente en dépendence des conditions géographiques.

Voilà un aperçu sommaire des travaux qui sont menés par l'Institut.

L'immense territoire soulève beaucoup de questions, la pratique d'agriculture donne beaucoup de problèmes. Quelques uns de ces problèmes sont étudiés par les institutions provinciales, mais en même temps on doit centraliser les études de beaucoup de problèmes parce que seulement par la centralisation de quelques études on peut recevoir la syntèse des faits.

Sans doute, concernant beaucoup de branches, la Russie doit étudier l'expérience des autres pays, mais en même temps il n'y a pas de doute que quelques résultats des travaux scientifiques, qui sont conduits par l'institut central et provincial de Russie qui ne sont pas trop locales, peuvent intéresser les investigateurs d'autres pays.

THE ORIGIN OF CULTIVATED PLANTS[1]

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In the studies of the variabilities of different Linnaean species of cultivated plants the author has determined that the separate Linnaean species are definite *systems* of forms and not an accidental collection of different varieties. The nearest systems are characterized by a similar system of phenotypical as well as genotypical variabilities. For different families of plants certain general schemes of variabilities have been arranged (these are for Gramineae, Papilionaceae, Cucurbitaceae, Cruciferae). In the composition of these systems it would appear that there are many missing links in the chain—links which ought to exist theoretically but which have not been found. In consequence, the author took up the study of the geographical centers of the origin of cultivated plants. A new differential systematico-geographical method for the determination of the centers of formation was established. This method consists in the following:

1. (*a*) In the differentiation of the plant into two separate Linnean species and genetical groups by the aid of different methods: morphological, systematic, hybridological, cytological, and by its reaction to parasites.

(*b*) In the determination of the areas occupied by these species, if possible, in remote times, when the means of communication were more complicated.

(*c*) In the determination of the composition of varieties (more exactly of the variable characters) of each species and of the general system of hereditary variation.

(*d*) In the study of distribution of different characters in the world and in the determination of the geographical centers of concentration of variability.

2. To be more exact, it is necessary to find the geographical centers of the concentration of the diversity of genetically allied species.

3. In the determination of the area of the diversity of the nearly allied wild varieties and species.

4. In the application of the methods of archeology, history, and linguistics on the basis of botanical data.

As a result of the application of this method it was found that wheat has three different geographical centers of origin. This determination was made after several expeditions to southwestern Asia, and after the study of numerous collections. The group of soft wheats has originated from southwestern Asia, in the regions of southeastern Afghanistan, northwestern India, and the neigh-

[1] Presented by title before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 20, 1926.

boring countries. Durum wheats have originated in the Mediterranean and especially in the northeastern part of Africa. *Triticum monococcum* has come chiefly from Asia Minor.

Cultivated barleys have come from two different centers; hulled varieties are native of Abyssinia, hulless varieties, of southeastern Asia.

Oats are of polyphilatic origin. Geographically the origin of cultivated oats is the following: (1) For *Avena bysantina* and *A. sterilis* it is the Mediterranean, chiefly northern Africa. (2) For *Avena abyssinica* and nearly allied varieties, Abyssinia. (3) *Avena strigosa*, *A. brevis*, *A. nudis-brevis* have originated from northwestern and western Europe. (4) *Avena sativa*, and *A. orientalis* are with difficulty referred to different regions. Several original varieties of *Avena sativa* were found in Transcaucasus and in China. (5) *Avena nuda*, large-grained varieties, are of Chinese origin.

Millet (*Panicum italicum* and *P. miliaceum*) have come from southeastern Asia.

Flax is of two-fold origin. Varieties with large seeds, large flowers, and large capsules are of Mediterranean origin. Other varieties with small seeds, small flowers, and small capsules, varieties cultivated for fiber as well as for oil, are concentrated in their diversity in southwestern Asia.

From these regions these varieties of flax spread into Europe and Siberia. Forms with a short period of vegetation went naturally to the North. Varieties with a long period of vegetation were naturally left in the South. According to the direct correlation between the short period of vegetation and the length of the stem, flax for fiber is cultivated in the northern regions of Europe and Asia. Varieties with a longer period of vegetation are cultivated in the south, chiefly for seeds.

The botanical-geographical study established that half of our ordinary field crops were formerly weeds among different crops in the south. Therefore, for the practical purpose of plant breeding it is necessary to look for the new varieties among the weeds of these crops.

Cultivated rye proved to have originated from weed rye, which even at present is a noxious weed in the sowings of wheat and winter barley in Turkestan, Persia, and Afghànistan.

Oats came into culture from the weeds which were found among ancient crops, chiefly among emmer and barleys. A curious fact is the great diversity of *Avena sativa*, found as a weed among separate patches of emmer in different countries.

The old crops—wheat and emmer—in their distribution to the north from their primitive centers of origin brought with them weeds into the cultures of the north; and in the severe conditions of this region these weeds became independent crops.

The author would divide all cultivated plants into two groups: one that has originated from weeds, such crops as oats, rye, many Cruciferae, and different vetches; the other consists of fundamental crops known only in cultivation.

The mountainous regions of Asia, northern Africa, southern Europe, and South America have proved to be the chief centers of the origin of cultivated plants.

In the study of the geographical distribution of different varieties and different species it has been found that there are different geographical regularities in their variabilities.

The Mediterranean region has given large-grained varieties of cereals, peas, flax, lentils, beans. On the contrary, the regions of southeastern, and southwestern Asia are characterized by the formation of small-grained varieties.

The chief centers of the origin of cultivated plants are: (1) the mountain regions of southwestern Asia; (2) the mountain regions of southeastern Asia; (3) the Mediterranean region; (4) Abyssinia; (5) the mountain regions of South America and Mexico.

By establishing the centers where the diversity of variation is concentrated, these botanical studies proved to be of the greatest importance for ascertaining the centers where are to be found the largest number of varieties necessary for plant breeding. Many new original forms were found, which obliged us to change our conceptions as to the diversity of many cultivated plants.

INTRODUCTION OF CULTIVATED PLANTS INTO THE UNION OF SOVIET SOCIALIST REPUBLICS (RUSSIA)[1]

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The Russian peasant, as far back as the ninth century A.D., cultivated some European and Asiatic crops, for instance: rye, wheat, oats, peas, flax, hemp, and millet. In Turkestan, Asiatic varieties of cotton, alfalfa, and some sorghums have been grown for centuries. Apples, pears, and grapes have been represented in greatly diversified forms.

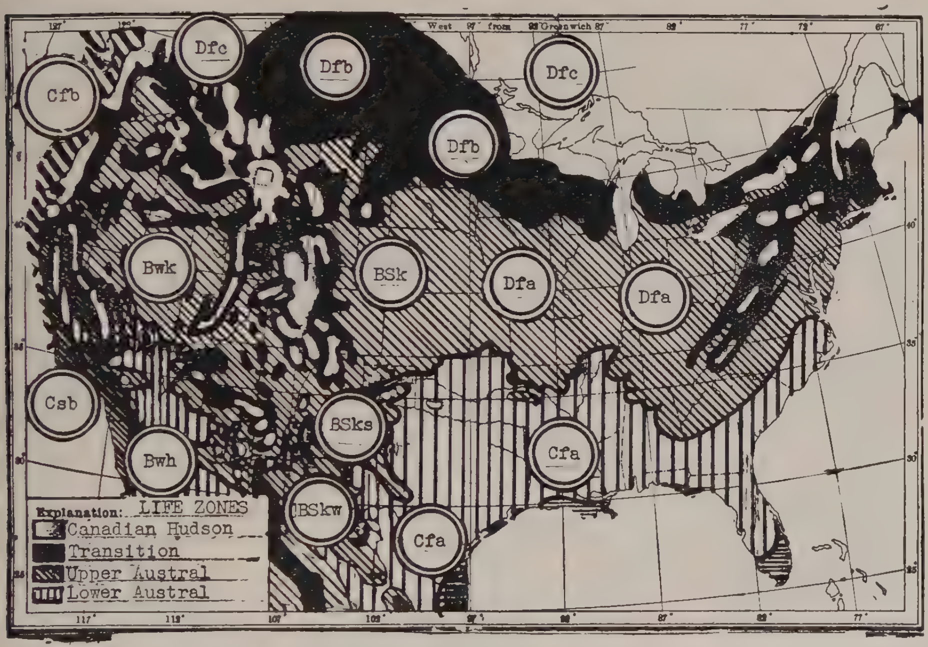

FIG. 1. Life Zones and Climate of North America. (See note at end of paper.)

The earliest introduced plant from America, adapted by the peasants, was sunflower, and somewhat later—tobacco and potatoes. American corn, Chile pepper, and tomatoes appeared at a later period, during the nineteenth century.

Toward the close of the last century America attracted Russian agronomists as a source from which new plants adaptable to the Russian climatic conditions were to be found, and systematic introduction of these plants began.

After the Russian Revolution, organized work of plant introduction has been undertaken through a special organization, namely: The Russian Agricultural

[1] Abstract of paper presented before the International Congress of Plant Sciences, Section of Agronomy, Ithaca, New York, Aug. 20, 1926.

Agency in America (formerly, Russian Bureau of Applied Botany of the Agricultural Scientific Committee).

A study by the author (during a trip through the United States in 1921) of results obtained from Russian field crops cultivated in this country stimulated

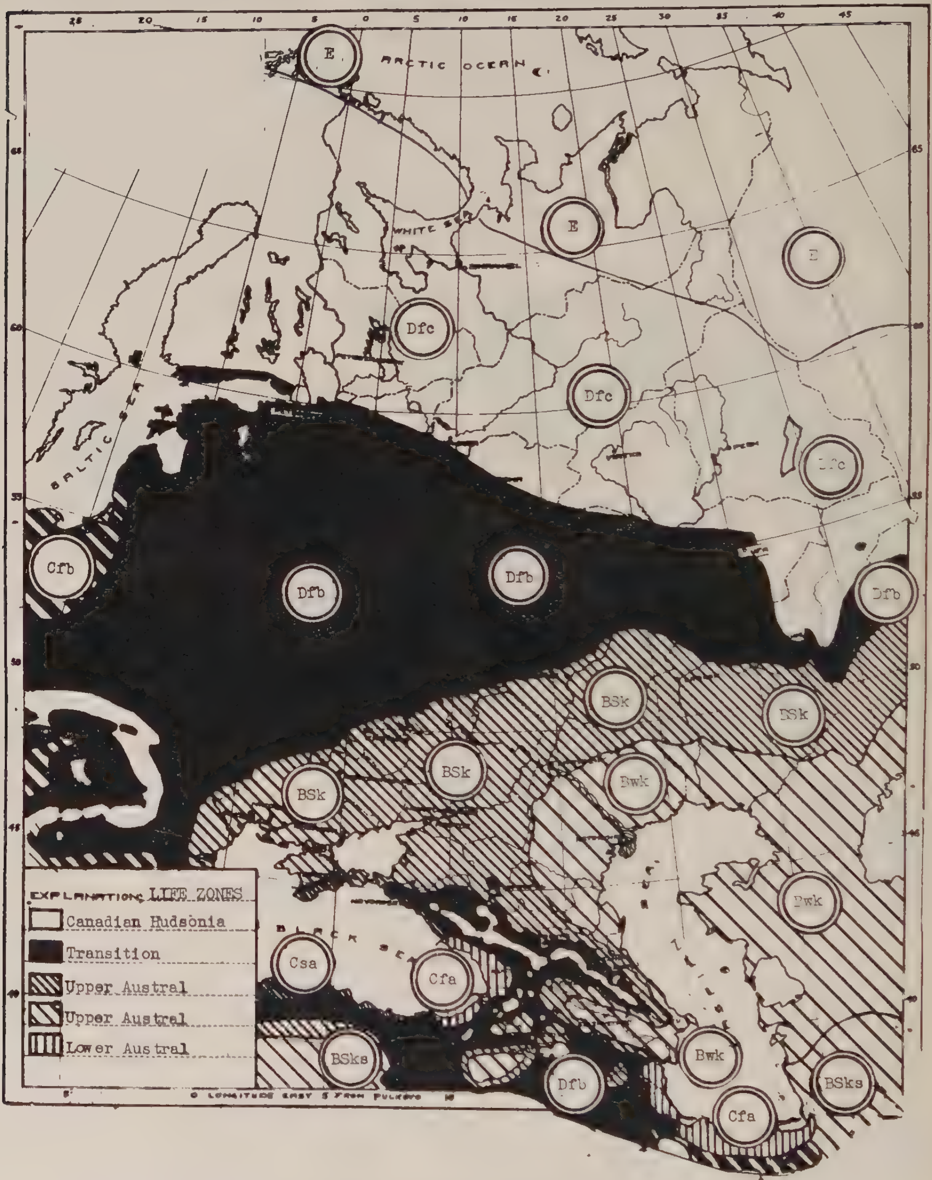

FIG. 2. Life Zones and Climate of European Russia. (See note at end of paper.)

me to attempt on a new basis the introduction of these crops on a large scale. This idea was enthusiastically received by Prof. N. I. Vavilov.

The theoretic basis of organized introduction has been established by comparing meteological maps of Russia with those of the United States. Life Zone maps of C. Hart Merriam, books on distribution of natural vegetation by B. E. Livingston, and classification of climates by Koeppen were of great value in our work.

The absence of maps of Russia showing frost-free periods presented an obstacle in the preparation of a special scheme of foreign plants and seeds introduction. No special maps of distribution of cultivated plants of Russia, with indications of varieties, have been used there prior to the year 1921, and such maps were later prepared by the All-Russian Institute of Applied Botany and New Cultures.

Organized introduction work up till the present time gave Russia the opportunity to obtain more than 10,000 samples of various seeds and plants from America, and these are now distributed all over the Union of Soviet Socialist Republics, through the All-Russian Institute of Applied Botany and New Cultures. Some of them were tested on the experimental plots from north to south and from east to west of the Union. Others are now recognized as valuable plant immigrants and are cultivated on a large scale.

Marquis wheat, Kitchener, Kota, Acme, wilt-resistant flax, Sudan grass, Grimm alfalfa, many varieties of sorghums, soy beans, and cow peas, as well as other plants selected and hybridized at the American experiment stations, have been added. Of especial value is the introduction of Sudan grass, Grimm alfalfa, Ivory King corn, also hardy varieties of Indian corn from Canada and the Northwest, most of which, hardy and drought-resistant, came from the Indian reservations of the United States.

Note. In the two comparative maps there are presented, first (Fig. 1), North America, as given by Dr. C. Hart Merriam—with some indications from Koeppen's book on the classification of climates; second (Fig. 2), on the same basis, with identical shading, I have outlined the regions in the European and Asiatic parts of the Union of Soviet Socialist Republics; thus emphasizing the corresponding climatic areas.

ON THE MORPHOLOGICAL AND PHYSIOLOGICAL VARIATION OF BACTERIA[1]

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Mr. Chairman, Ladies and Gentlemen.

Being neither a plant scientist nor a bacteriologist, I must apologize for my boldness in addressing this meeting. As a human pathologist, I have, however, been confronted so many times with the problem of bacterial variation, that I have found it worth a closer study in spite of my lack of proficiency in the above mentioned sciences.

The first difficulty we encounter when approaching our problem is that nobody has given us an acceptable definition of bacteria. It is evident that many of the microorganisms called bacteria are related to very different groups of the lower forms of living organsims. They have often only superficial characters in common, as, for example, the capacity of causing disease. Hence we must always have in mind the danger of undue generalization.

The bacteria are divided into orders and families which have, however, rather different limitations with different authors, causing much confusion. The most prominent groups, at least in medical bacteriology, are the cocci, the bacilli and the spirilli. At first I shall devote myself to the bacilli. These are supposed to be rod-like in shape and very uniform in size and appearance. A closer scrutiny of such an organism, for example *Bacillus coli*, which nobody denies being both a *Bacterium* and a *Bacillus*, reveals, however, that it has many forms which are anything but rod-like. During the last fifty years these aberrant forms have been the subject of discussion, dividing bacteriologists into two parties; the one seeing in the circumstance mentioned only involution forms, the other claiming that the so-called involution forms are real vegetative cells or even cells specialized for the reproduction of the species. I do not think that the position of the latter party is well accepted, but scarcely anyone now denies that a young fresh culture of *B. coli* or of *B. typhosus*, and many other bacteria, often contains a great variety of forms that are not involution forms. Hundreds of papers illustrate this. Exceptionally beautiful pictures may be seen in Hort's[2] papers (Pl. I, Fig. 1). Even more conspicuous are these forms when we look at *Bacillus diphtheriae* in a hanging drop preparation. The many peculiar cells are here impossible to overlook and this fact has caused bacteriologists to give this micro-

[1] Presented before the International Congress of Plant Sciences, Section of Bacteriology, Ithaca, New York, Aug. 19, 1926.

[2] British Med. Journal **1**: p. 571. 1917.

organism the name of *Corynebacterium*, thus indicating its close relation to the higher fungi, that is, the Hyphomycetes.

Before going further, it will be necessary to make clear what shape the aberrant forms can take. Hort's pictures of *B. coli* and *B. typhosus* show globular, oval, and egg-shaped cells; also filaments, some of them with real branching. To the filaments are often attached egg-shaped or oval cells, which can only have been produced by direct budding from the filaments and they are, evidently, not secondary adherences. Some of the cells are sausage-like or rod-shaped, others have a diverse appearance difficult to describe. If we consider *Bacillus diphtheriae* we shall find, first, the forms just mentioned, and in addition some forms of great theoretical interest. The branching filaments will be found to be divided by cross-partitions, and the round bodies will often be seen in a budding stage. The budding and branching has been followed up in warm-stage preparations by Hill[3], Bergstrand, and others.

All the forms mentioned (Pl. I, Fig. 2) are familiar to the mycologist who sees them in the Saccharomycetes and other real fungi. The following quotation from Hansen illustrates this clearly: "The starting point for experiments with yeast," says Hansen, "must be an absolutely pure culture originating from a single cell. Even in a culture of this kind the cells will be found to be dissimilar. One cell does not resemble the other. This applies also to species which, like *Saccharomyces cerevisiae* I, occur in the most uniform individual forms. The shape of the saccharomycetic cell may change from regularly globular or oval, through more or less pointedly egg-shaped, to oblong sausage-shaped, or to rod-shaped. The cell may branch and appear in the most singular shapes. This state of affairs may be differently accentuated in different species. *Each individual can give rise to a colony consisting of all the forms mentioned.*" *Later on he indicates how the yeast may have a mould-like growth and show a mycelium with distinct cross-partitions.*

The resemblance between the polymorphism of *Saccharomyces* and that of *B. coli* and *B. diphtheriae* is striking. Thus it seems to me most natural to see in the differently formed cells in the culture of a bacillus only normal vegetative cells, just as we view, without astonishment, such cells in a broth containing a species of *Saccharomyces*. The groups of microorganisms just mentioned have not only an external similarity, but a much closer likeness, since the individual cells in both groups, broadly speaking, have the same structure, as especially A. Meyer[4] points out. The only noticeable difference is the absence of a distinct nucleus in the bacterial cell. That does not mean so much, however, since it is very difficult to prove or disprove the character of all the granular bodies which can be found in these cells. Even in the saccharomycetic cell the nucleus is all but easy to find.

The appearance of a culture of *Saccharomyces* is dependent on several things, and one factor is the composition of the substrate. This phenomenon is, in its turn, well known in the higher plants. A *Taraxacum* growing in the moun-

[3] Journ. of Med. Research 7: p. 115. 1902.

[4] Die Zelle der Bakterien. Jena, 1912.

tains is quite different from the same plant developing in a valley. As for *B. coli*, it has a rather uniform and similar appearance cultivated in ordinary laboratory substrates, but it needs only to be grown under somewhat changed conditions in order to assume another aspect. In cystitis caused by *B. coli*, to take an example from human pathology, the bacterium will sometimes take the form of long filaments which, transferred to common broth, change into short rods or oval forms. Once more I shall quote Hansen to show that exactly the same thing happens in *Saccharomyces*. Speaking of the mould-like growth of yeast he says: "If these mould-like growths be transferred to wort, cells are developed which gradually return to their original state." And later in his paper one reads the following: "As a rule, a certain relation is maintained between the oval and the sausage-shaped cells of a species. In forms belonging to *Saccharomyces cerevisiae* and *Saccharomyces ellipsoides* the oval cells are predominant. The sausage-shaped cells on the other hand are in majority in species belonging to *Saccharomyces pastorianus*. This is the rule, but there are occasions when a definite cell-form can continue to reproduce itself through several generations in a more marked manner, so that the new colonies get quite a heterogeneous character."

The aberrant forms in a culture of *Saccharomyces* have never been stamped as involution forms. The reason why the involution forms have played such a great rôle in bacteriology is the fact that this science has been developed to a great extent by medical bacteriologists, and these have been too much interested in the task of furnishing morphogical data which could be used for the direct identification of bacteria from exudates or pathological tissue. A *Bacillus diphtheriae* is known and identified as a rod when taken from the throat and grown 18 hours on Löffler serum. Hence all forms which are not rods have been, if not overlooked, at least neglected. Even in the modern text books of bacteriology the *Bacillus diphtheriae* is represented as a rod-like thing with "polar granules."

On the other hand, from my point of view, Löhnis,[5] Hort, and others have gone too far, when they see in the different forms of a culture links of a chain, which they call the "Life-cycles of Bacteria." This seems to me quite as impossible of comprehension as if somebody were to maintain that the globular, oval, and sausage-shaped cells in a saccharomycetic culture form a "Cycle of Development."

In the foregoing it has been pointed out that the appearance of a bacterial culture is in a certain degree dependent on the composition of the substrate in use. *B. coli* can be made to grow as long filaments or as short rods, if only a suitable medium is chosen. Up to this point we are evidently dealing with modifications effected by new enviroments, well known in plants and animals. The same applies, of course, also to those conditions when a bacterium changes its biological character by transfer to a different medium. The influence of the medium on the whole chemism of bacteria has been shown very nicely by Dawson[6]

[5] Memoirs of the National Academy of Sciences, Washington, 1921.

[6] Journal of Bact. **4**: p. 133. 1919.

working with *B. coli.* He bred an old laboratory strain of this bacterium for 200 generations on 8 different media of known composition, finally collected the growths of nine days and made chemical and serological determinations. Both chemically and serologically the 8 new "strains" differed greatly from the mother culture and among each other. Immune sera prepared by 4 of the "strains" agglutinated the homologous "strain" to the highest titer. This means a chemical differentiation great enough to give a specific reaction even in the body of an animal.

But there is a great difference between bacteria and higher organisms in their behavior when the particular environmental factor is withdrawn. In higher animals and plants the modifications then immediately vanish: not so in bacteria. On the contrary, the characters acquired by the influence of the acting principle in the substrate are often held with great tenacity for thousands of generations after the removal of this agent. Let us once more take an example from *B. coli.* Fabry[7] showed that if this bacterium is seeded from a common broth culture into a substrate containing phenol it loses the ability to form indol. This new character is retained during many months, even if the bacteria are grown on phenol-free media or transplanted to animals. The induced or, to use the language of Adami[8], impressed characters have been made hereditary. In this case the creation of new biological features was combined with morphological changes, but sometimes it is not.

This phenomenon is known and studied in the protozoa. If fuchsin is injected into a mouse infected with a *Trypanosoma*, the microorganisms vanish from the blood, provided that the dose is a suitable one. Should the dose be too small, some of the Trypanosomas will survive and reproduce new individuals. Repeated injections finally fail to have any influence on the microorganism. A fuchsin resistant strain is formed, which holds this character, even if transferred from mouse to mouse for many years. This induced character disappears at once, however, if the microorganisms are allowed to conjugate, which last may happen if they are transplanted to the mouse-louse.

The first to direct a more general interest to such induced strains or variants in bacteria was Massina[9] (1907). Before him several authors had, indeed, made analogous observations as, for example, Brunton, Rodet, Peckham, Grassberger, Klotz, and Twort. Massina found a strain of *B. coli* growing with colorless colonies in the endoplate, that is, without power to ferment the sugar in this substrate. From time to time he saw small red spots appear in the white colonies of the original culture. These spots contained bacteria endowed with the faculty of fermenting the lactose, thus coloring the plates red, which ability they retained even if transferred to sugar-free substrates. It was evident that the formation of the new strain was due to the influence of sugar. Analogous conditions have been demonstrated in other bacteria to a great extent.

[7] Compt. rend. d. la Soc. d. Biologie **85**: p. 844. 1921.

[8] Medical Contribution to the Study of Evolution, London, 1918.

[9] Archiv. f. Hygiene **61**: p. 250. 1907; Zeitschrift f. ind. Abstammungs-und Vererbungslehre **2**: p. 90. 1909.

The often observed appearance on the surface of the mother-colony of spot-like daughter colonies containing individuals forming a quite different strain is doubtless a phenomenon of this kind, caused by the metabolic change in the ageing culture. Henrici will deal with this matter, I feel sure, in the paper he is just going to read. Figure 3 (Pl. II) demonstrates such daughter colonies appearing on the colonies of a strain of *B. coli*, isolated from a single cell in an old laboratory culture. The same thing will be seen in Figure 4 (Pl. II), which represents another strain, isolated in the same way from the same culture. The 2 strains showed great differences, the one being non-motile, the other motile. Further study of the secondary strains obtained from the daughter colonies revealed new variations, but those bacilli originating from the motile one did not lose their motility, and *vice versa*. It seems as if a number of bacteria, such as, for example, *B. coli B. typhosus*, and *B. proteus*, ordinarily appear in 2 principle forms, having some very constant characters, one of which is motility or non-motility. The older literatute on this subject will be found in a paper by Nyberg, whereas Weil[10] and his co-workers have dealt with the problem chiefly from the serological point of view.

The induced variants may be very stable or more or less labile. Wolf working with *B. prodigiosus* obtained strains growing as white colonies at room temperature, whereas the ordinary *B. prodigiosus* is red. Some of the white strains were irreversible, while on the contrary others soon separated red colonies again. The laws governing this variation are at the present time practically unknown. To speak of mutations, as a great many authors do, seems to me provoking and unnecessary.

Bearing in mind the above mentioned state of things with protozoa, we must seriously consider the question of a sexual reproduction even with Bacteria. Many authors claim that they have seen not only stages analogous to those associated in higher fungi with a sexual process, but also real conjugation.

Almquist describes bacterial plasmodia, and Löhnis and Smith talk of a "symplastic stage," in which the bacteria transform to amorphous masses. The latter 2 authors, especially, have considered this stage most important as the beginning and end of a life-cycle of bacteria. As to the nature of these forms, whose importance also is advocated by Jones.[11] I will offer the suggestion that they perhaps are the same things which have been seen before and called "Zoogloea"; that is, clusters of degenerated bacteria kept together by mucus. It is true that these masses contain living matter, because new cultures can be obtained from them. But how can one prove that the new growth is not started from common vegetative cells enclosed in the mucus?

Quite a number of observers have seen in different bacteria spheroidal, thick-walled bodies, which have been much discussed. Speaking of *B. azotobacter*, Jones writes as follows: "In cultures more than 14 days old, large spherical, thick-walled cells are common. These appear to be resting cells or arthrospores. On transference to fresh media these thick-walled cells germinate, the

[10] (Lit. by Felix) Journal of Immunology 9: p. 115. 1924.
[11] Journal of Bact. 5: p. 425. 1920.

cell plasma emerging from the thick wall as a large rod which at once proceeds to multiply by fission." I have seen and studied these bodies with *Vibrio cholerae*, *B. diphtheriae* (Pl. II, Fig. 5), and some other bacteria, and I fully agree with Jones. Never have I been able to trace the slightest sign indicating that these cells might be organs of sexual reproduction. On the contrary, their very form indicates their nature as chlamydospores, or "resting cells," to use an indifferent name (Pl. II. Fig. 6). Almquist[12] (Pl. III. Fig. 7), Hort, Jones and Mellon,[13] and others claim, however, that these bodies, or at least bodies of similar appearance, contain within their walls a number of small cells that become free by the bursting of the wall. Mellon only saw this process in the living cell and he was working with a rather unknown fuso-spirillary microorganism. Hence one can always make the objection that he was handling a real fungus and not a bacterium. The same applies to Tunicliff and Jackson,[14] who have undoubtedly isolated from the human throat a bacillus-like microorganism with real reproductive organs. Such formations mean a sexual process. As mentioned before, some authors, especially Löhnis, Smith, Mellon, and others believe that they have actually seen this process; that is, a union of two or more cells. Their pictures are striking, but I am much inclined to doubt, because it is very common that bacteria do not separate completely by fission, but adhere to each other sometimes forming the most peculiar forms.

Hitherto we have spoken principally of bacilli because most of the work in these lines is done with such microorgansims as material. There is no doubt, however, that what is said above is applicable to most bacteria. As for the cocci, it may be that they are uniform morphologically, but if one isolates the individuals of a colony it will be found that they differ biologically. Felton and Daugherty, working with cultures of pneumococci derived from single cells, observed that if individual cells of such a culture were isolated they gave rise to cultures of varying virulence.

To sum up our knowledge of bacterial variation the following can be said:

(1) In a culture the individuals are not absolutely similiar, even if the strain is derived from one single cell.

(2) The individuals may differ both morphologically and biologically.

(3) If the individuals in a culture be isolated they may give rise to daughter-cultures of different qualities, even if kept under the same conditions.

(4) A bacterial population is influenced by environmental factors, and if the influence is allowed to operate for a sufficiently long time: that is, during many generations, the new characters will be hereditarily fixed.

(5) The characteristic of a bacterial cell is dependent both on the culture medium and on its own hereditary tendencies.

(6) As induced characters are often held with the greatest tenacity, new so-called "strains" may easily be experimentally formed.

[12] Zeitschr. f. Hygiene **83**: p. 1. 1917.
[13] Journal of Bact. **4**: 1919; **10**: 1925.
[14] Journal of Inf. Diseases **36**: p. 430. 1925.

The problem of bacterial variation has a bearing on many important questions. The ability of these microorganisms to adapt themselves to different conditions is naturally related to the origin of diseases in plants, animals, and man. Saprophytic bacteria have been experimentally transformed into pathogenic ones by many experimenters. The interested may consult Adami's "Medical Contributions to the Study of Evolution."

Variation of the pathogenic agent probably plays a great rôle also for the character of the epidemic diseases or, to use another word, for the "genius epidemicus."

The last great influenza pandemic has furnished facts which, I think, can be used as an example. Without the pretension of having anything new to demonstrate, I will show you some rather striking figures from the influenza epidemics in my own country.

Figure 8 (Pl. III) is a map of Sweden, with the country divided into the 24 administrative districts. Each of these is provided with a figure denoting the month during which the influenza cases reached their maximum in 1920. It will be seen that this occurred in February in the northern and southwestern parts of the country. A number of districts running northwest-southwest through the middle of the country did not show the maximum until April. These districts are bounded on both sides by a belt where the maximum occurred during March. If, on the other hand, we look at the map for 1922, (Pl. III, Fig. 9), we find that the southeastern part of the country had its maximum in January and the remainder in February. Now, these conditions can only have one explanation; namely, that the influenza migrated over the country. These epidemics have therefore not occurred from influenza breaking out here and there, but through the infection spreading from one, respectively, two points. In 1920, as well as in 1922, the District of Upsala differed from its surroundings. It might be thought that this would render the conclusions drawn rather unreliable. However, the phenomenon can be easily explained. The figures of this district are determined by the figures of the town of Upsala, a small university town, where, when the terms begin in January, there is an influx of 2500 students from different parts of the country.

A remarkable circumstance is that the epidemic of 1920 proceeded at a slower rate than in 1922. As the communications were the same both years, this may only be explained by the fact that more people contracted the disease in 1922, which means that it was then more contagious. In spite of this, deaths were much more frequent in 1920, namely, 2853 as against 1970 in 1922.

Evidently we are here dealing with two variants of the virus, one very infectious, but rather mild as to its character, and another less infectious, but with great capacity to produce severe forms of disease.

It would be very alluring to go deeper into this question, the statistical material being rich and interesting, but the limited time at my disposal forces me to confine myself to the suggestions already made here.

Finally, it may be mentioned that the study of bacterial variation certainly will change our conception of what is called "species" and "type" in bacteria.

One single example will suffice to show this. For a long time the bacterium causing lobar pneumonia has been called *Pneumococcus* or *Streptococcus lanceolatus*, and regarded as a fixed species, and this might even be divided into equally fixed sub-species or varieties. The same has been the case with *Streptococcus viridans*, causing a certain well defined heart disease called endocarditis lenta. In the course of time, however, many observers (lit. by Rosenow,[15] Wolff[16] and Lehmann[17]) have found that the morphological and biological differences between the two bacteria are not reliable, since the characteristics of the former can be impressed on the latter and *vice versa*. The only difference that remains is that *Streptococcus viridans* does not agglutinate in type-specific pneumococcus serum. But supposing the specific agglutinability of the pneumococcus is only a quality acquired by the microoganism when adapted to cause pneumonia? If so, this character must be a passing one, and possibly the pneumococcus could be forced to take on even the serological characters of a *Streptococcus viridans*. In my laboratory Görander has shown this to be the case. By simple methods, which nevertheless would take too much time to describe here, he forced type-specific pneumococci to take on all the characteristics of a typical *S. viridans*. During the procedure the pneumococci lost their specific agglutinability. Sera prepared with the pneumococci and with the *S. viridans*, now showed cross agglutination to the same very high titer, and by absorption tests both bacteria absorbed completely not only the homologous, but also the heterologous agglutinins. The same experiments were repeated with virulent pneumococci having all their characteristics, except specific agglutinability, which was eliminated by repeated transfers from mouse to mouse; also with *S. viridans* cultivated to obtain all the characters of virulent pneumococci. The result was exactly the same and is demonstrated by the table.

TABLE 1. AGGLUTINATION AND CROSS AGGLUTINATION

Serum dilution	1/10	1/20	1/40	1/80
P 0.2 cc.+ps	+++	++	++	+
P " +vs	+++	++	+	+
V " +vs	+++	++	+	+
V " +ps	+++	+++	++	+
V " +ns	–	–	–	–
P " +ns	–	–	–	–

Explanations of abbreviations used are as follows:
P = Emulsion of two times washed pneumococci obtained by centrifuging a broth culture.
V = Emulsion of *Streptococcus viridans* prepared in the same way.
ps = Pneumococcus serum.
vs = Viridans serum.
ns = Normal serum.

[15] Journal of Inf. Diseases **14:** p. 1. 1914.
[16] Virchow's Archiv. **244:** p. 97. 1923.
[17] Deutsches Archiv. f. Klin. Medizin **150:** p. 127. 1926.

TABLE 2. ABSORPTION TEST
(Explanations as in preceding table)

Tube 1	P 0.4 cc.+ps	0.4 cc.+4cc.	Na Cl-solution
Tube 2	P " +vs	" + "	" "
Tube 3	V " +vs	" + "	" "
Tube 4	V " +ps	" + "	" "

From these tubes centrifugates were prepared; these were used in Table 3, and are designated as follows:

Centrifugate from tube 1 = c_1; Centrifugate from tube 3 = c_3;
" " " 2 = c_2; " " " 4 = c_4.

TABLE 3. AGGLUTINATION WITH THE CENTRIFUGATES

Serum dilution	1/10	1/20	1/40	1/80
P 0.2 cc.+c_1	+	+	–	–
V " +c_1	+	–	–	–
P " +c_2	+	–	–	–
V " +c_2	+	+	–	–
P " +c_3	+	+	–	–
V " +c_3	+	–	–	–
P " +c_4	+	+	–	–
V " +c_4	+(+)	–	–	–

It is evident that V+C_2 does not give a much stronger agglutination than P+C_2, which it should do, since the serum is a viridans serum saturated with pneumococci. Hence the pneumococci have been able to remove not only their own heterologous agglutinins, but also the homologous ones. The same applies to P+C_4.

This investigation seems to me to indicate that *Pneumococcus lanceolatus* and *Streptococcus viridans* are only different manifestation of the same thing, a theory which is not new, but has been put forward many a time before.

Being at the end of my paper, I wish to express my great appreciation of the honor conferred on me by the invitation to appear before this audience, and I hope you will overlook my having dwelt perhaps too long in that doubtful borderland of our knowledge called the variation of bacteria.

Explanation of Plates

PLATE I

Fig. 1. *Bacillus coli communis* according to Hort. In Hort's paper this drawing is verified by photomicrographs. Brit. med. Journal **1**: (1917).

Fig. 2. India-ink preparation of diphtheria bacilli from a 24-hours-old culture. Photomicrograph. After Bergstrand, Acta Otolaryngologica **1**: (1918).

PLATE II

Fig. 3. A 1-month-old agar plate of a non-motile strain of *B. coli* demonstrating secondary colonies in the form of droplets. Bergstrand, Journal of Bact. **8**: (1923).

Fig. 4. A 1-month-old agar plate of a motile strain of *B. coli* derived from the same old laboratory culture as the non-motile strain. The secondary colonies are few and have the form of rings. Bergstrand, Journal of Bact. **8**: (1922).

Fig. 5. Round, thickwalled body from an old culture of *Bacillus diphtheriae* supposed to be a resting cell. Bergstrand, Acta Otolaryngologica **1**: (1918).

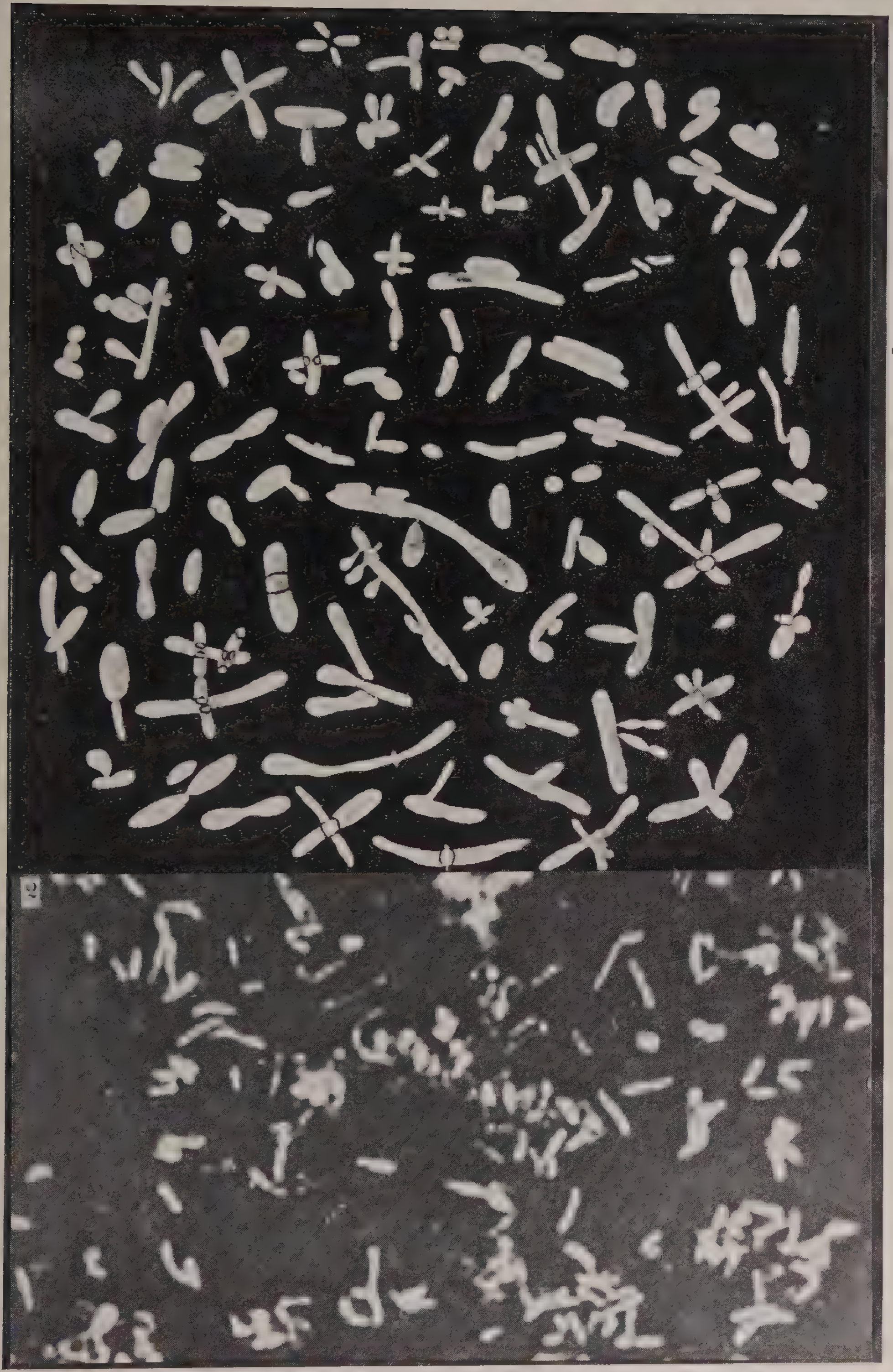
Fig. 6. Preparation from a microorganism isolated by Mellon from a case of fuso-spirillary infection. Development of a chlamydospore in the middle of a filament. The swelling of the thread, the thickening of the wall, and the gathering of the protoplasm to the spore are clearly seen. Bergstrand, Journal of Bact. **8** (4): (1923).

PLATE III

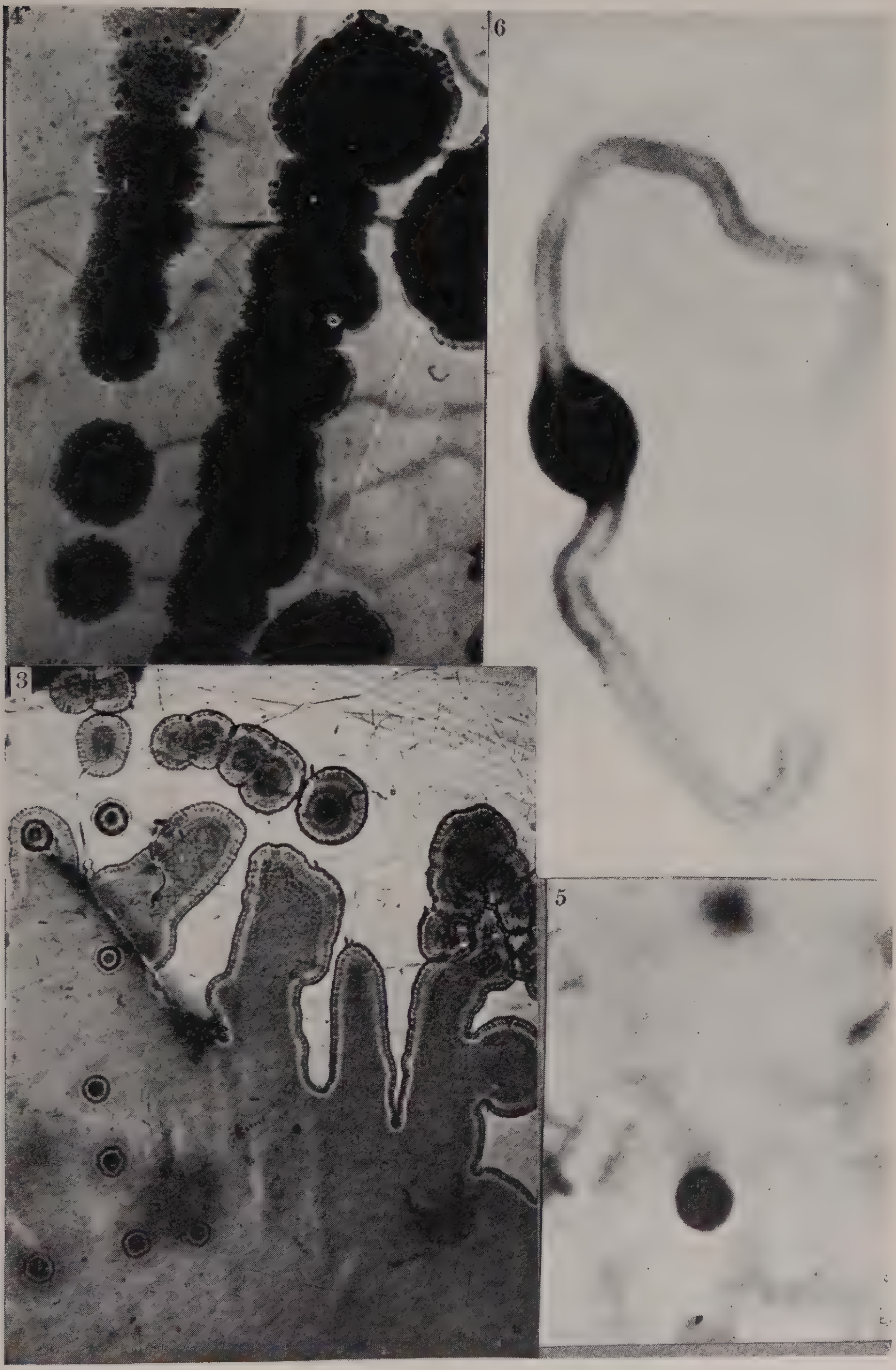
Fig. 7. *Bacillus typhosus* according to Almquist, Zeitschr. f. Hyg. Bd. **83**: (1917).

Fig. 8. Map of Sweden with the 24 administrative districts. Each district is marked by a figure denoting the month during which the influenza cases reached their maximum.

Fig. 9. Map of Sweden with the 24 administrative districts. The figure marked on each district denotes the month during which the influenza cases reached their maximum.



BERGSTRAND: VARIATION OF BACTERIA



BERGSTRAND: VARIATION OF BACTERIA

BERGSTRAND: VARIATION OF BACTERIA

MORPHOLOGICAL VARIATION AND THE RATE OF GROWTH OF BACTERIA[1]

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A statement by D'Arcy Thompson ('17) in his work "Growth and Form" expresses the viewpoint from which I have approached the problem of morphologic variation in bacteria. He says: "It is perfectly true that all changes in form, inasmuch as they necessarily involve changes of actual or relative magnitude, may in a sense be properly looked upon as phenomena of growth, and it is also true since the movement of matter must always involve an element of time, that in all cases the *rate* of growth is a phenomenon to be considered." This viewpoint is somewhat different from that of those who have preceded me in this field of investigation, for both monomorphists and pleomorphists have paid but little attention to the factor of time as related to cell changes, the former being satisfied with standard observations of 24-hour cultures, the latter attempting to patch together life cycles from more or less isolated observations without regard to the phase of growth in the culture. In this paper I shall try to show that there is a very great similarity between the simple one-celled microorganisms and the large multicellular organisms in the laws which govern their growth and the transformations of their cells; that the cells of bacteria exhibit during the growth of a culture a series of changes analogous to the changes of those of a multicellular organism during the growth of an individual, presenting in turn a young or embryonic form, a mature or adult form, and a senescent or dying form; and that these variations are dependent upon the metabolic rate, the change from one type to the other occurring at points of inflection in the growth curve.

Minot ('13) has coined the word "cytomorphosis" to designate the progressive cell changes which occur in the cells of a multicellular organism during growth and senescence, and this term, I believe, more exactly expresses the nature of the morphologic variations which occur in bacteria than does the term "life cycle." What I mean by cytomorphosis in bacteria can be illustrated by observing the cells of *Bacillus megatherium* taken at various intervals from an agar slant culture. You will see that the cells used for seeding, taken from an old culture, are in part spores, and in part small irregular granular cells which are always found in old cultures and which we commonly designate as involution forms. As the culture begins to grow, both of these types germinate, that is,

[1] Presented before the International Congress of Plant Sciences, Section of Bacteriology, Ithaca, New York, Aug. 19, 1926.

they become converted into a new cell type characteristic of the period of active growth; both the spores and the involution forms enlarge and assume a sausage form; the granules in the cells almost disappear. After the culture has reached its maximum growth, the cells again become smaller and filled with granules, and many of them develop spores which become progressively more numerous as the culture ages. From 48 hours on there appear more and more irregular, peculiar cell forms, that is, forms with lateral buddings, with swollen ends, and a few branching cells. There is then a progressive change in the cell structure from the moment of seeding until the culture is dead, and although these changes are continuous we can readily recognize three distinct phases in the process: the occurrence of large cells without granules during the period of active growth; the occurrence of small cells with granules (and the development of spores), after the culture has stopped growing; and the development of irregular forms during the time when the culture is dying.

To establish clearly the relationship of these cell types to the phase of growth, we must determine the rate of growth and death by counting the number of cells at regular time intervals. When this is done and the values so obtained are plotted against time, we get the characteristic curve of growth. Such growth curves of bacteria have generally been plotted by using the logarithms of the numbers of cells as the ordinates, since such a curve shows the *proportional* change in numbers and the slope of the curve will express the *rate* of growth or death. At first there is no change in the number of cells, they remain dormant for a short period, then they begin to grow and increase in numbers at an increasing rate until a maximum rate of growth is attained, which is then maintained for a period of time, after which the rate of growth decreases until the numbers of cells remain constant. Following this resting period, death begins and proceeds at an increasing rate until a maximum death rate is attained, which is then generally maintained until the culture is dead. We can thus recognize four distinct phases: an initial resting period, a logarithmic growth phase, a resting phase, and a logarithmic death phase, with three points of inflection between these phases.

That there is a marked similarity in this phenomenon of growth in individuals and in populations was recently pointed out by Raymond Pearl ('25) in his work "The Biology of Population Growth." The growth curves for a multicellular plant, the cucumber; a multicellular animal, the white rat; a population of one-celled organisms, yeast; a population of multicellular organisms, fruit flies; and the growth of a part of a multicellular organism, the regenerating tadpole's tail, all have the same general form, and the same empirical formula will serve to express them all. Pearl suggests that, in general, the same laws govern growth whether it be of an individual, a part of an individual, or a population of individuals. These curves, as far as they go, are the same as those for cultures of bacteria.

Since the most striking changes in the cells during the period of active growth are changes in size, the correlation of the cell changes with the rate of growth during the logarithmic growth phase can be most readily determined by measur-

ing the size of the cells. This has been done in a number of cultures of *Bacillus megatherium*. After an initial resting period the cells begin to increase in size and reach a maximum size at about the point when the growth rate begins to decrease, that is, at the point of inflection between the phases of accelerating growth and of negative acceleration in growth. After this maximum size is attained, they rapidly decrease again to the original size. A high positive correlation between the size of the cells and the rate of growth can doubtless be established. This marked increase in the size of the cells during the growing period is common to most species of bacteria. It was first observed by Clark and Ruehl ('19). They found in the colon typhoid group, with the organisms of influenza and whooping-cough, with the spore-bearing bacilli, with the cholera vibrio, and with the various cocci, in each case a regular increase in size during the early hours of growth. The diphtheria and diphtheroid bacteria, on the other hand, showed a marked decrease, rather than increase.

Clark and Ruehl explained this increase in size of the cells as being due simply to the growth of the organisms preparatory to division, but this explanation is quite inadequate for several reasons. If this were the case, it should be observed in all organisms including the diphtheria group. The increase in size should never be more than one and one-half times that of the organisms inoculated, assuming that each organism will double in size before it divides, and that in any sample there will be just as many cells recently divided as cells about to divide. But, as a matter of fact, with *B. megatherium* I have found that the maximum size attained was approximately six times that of the cells introduced ('26). By observing developing microcolonies, it may be seen that the cells generally reach more than twice the original size before they divide, and that in each succeeding division a larger size is attained. These changes in cell size are also correlated with other changes in structure. Those species of bacteria which possess intracellular granules of one kind or another lose them during the period of active growth. The affinity of the cells for aniline dyes becomes greatly increased with all species, the cells stain much more intensely during the early hours of growth than they do at a later period. Moreover, the changes in cell structure are accompanied by physiologic changes; the young cells are more susceptible to the action of injurious agents, as has been shown by Sherman and Albus ('23). This is also true of the young or embryonic cells of a multicellular organism, as has been demonstrated by Child ('15), and it seems to be a general law. There is considerable evidence that these young cells in cultures are better antigens than are old cells, that they possess a higher degree of virulence, and are less susceptible to antibody action. Both in structure and in function then, the cells found in the active growth period differ from the cells in a resting culture, and may be looked upon as a special type, an embryonic form in every sense analogous to the embryonic forms of the cells of a plant or an animal.

I have pointed out that the degree of change in size is correlated with the rate of growth. This can be more clearly demonstrated by modifying the conditions of the culture so that the rate of growth is altered, which should lead to a corresponding change in the degree of variation in size. This may be done

in several ways. The rate of growth is dependent in part upon the size of the initial seeding, that is, upon the number of cells introduced into each unit volume of medium. This also seems to be a general biologic law. In curves showing the rate of growth plotted against the initial size of the population in a population of fruit flies—taken from the work of Raymond Pearl ('25)—and in a population of yeast—taken from data supplied by Brown ('05)—the 2 curves have the same form, and throughout a certain portion of the range the rate of growth is inversely proportional to the initial seeding. The smaller the number of cells introduced the greater is the rate of growth. The same thing is true in the case of the tadpoles' tails, the initial seeding is represented by the size of the stump left after amputation. The smaller the stump, the higher is the actual rate of regeneration (Durbin '09).

The rate of growth may also be modified by the amount of food-stuff available to the organisms. As far as I know, there are no data available for higher organisms, but with bacteria it can be shown that the yield of a culture, that is, the maximum number of cells attained, is proportional to the concentration of food-stuff in the medium. The higher the concentration of food-stuff in the medium, the greater is the yield of the culture. Since the maximum yield is attained at nearly the same time, in media of different concentrations, the rate of growth must be proportionately greater the higher the concentration of nutrients. The size of the seeding and the concentration of the nutrients then bear a reciprocal relationship to each other. Where the concentration of the nutrients is varied, the cultures all start from the same point but reach progressively higher levels. The pitch of the curve for the logarithm of the number of cells becomes steeper as the medium becomes more concentrated in food-stuffs. With different size seedings, the curves start from different levels, but all come to the same maximum. The pitch of the curve becomes progressively steeper with smaller seedings.

Now if the size of the cells is correlated with the rate of growth, the cells should be larger in those cultures which are most lightly seeded and in those cultures which have the greatest amount of nutrient. This was actually found to be the case in cultures of *B. megatherium*. Where the nutrient is progressively diluted, we find that the maximum size of the cells becomes progressively smaller. With a heavily inoculated medium the cells do not become as large as in a lightly seeded medium (Henrici '21, '24).

The rate of growth is also dependent in part upon the age of the cells used for seeding. This has been shown clearly by Chesney ('16). When transferred during the initial resting phase, the cells remain dormant a short time in the new medium. When transferred during the active growth phase, the initial rate of growth becomes increasingly greater with the age of the parent culture. When transferred during the resting phase, the cells again remain dormant while in the new medium. The age of the parent culture has a similar effect upon the size of the cells during the growth period. When transferred after 2 hours of growth, the cells reach a somewhat larger size than they did in the parent culture, but when transferred at 4 hours, they reach a still larger size.

When transferred at a time when the cells in the parent culture were decreasing in size, they proceed to increase again and reach a slightly higher maximum. When transferred at a moment when the parent culture had reached its original size, the cells in the new medium show a slight lag before they begin to increase, and the maximum is no greater than that in the parent culture (Henrici '24).

It is quite clear, then, that the degree of change in the cells is definitely correlated with the rate of growth as measured by cell division. This must be true for a multicellular organism also. Child ('15) has shown that the degree of cell differentiation in a multicellular organism varies with the metabolic rate in the different parts of that organism. We have, however, no quantitative data by which the degree of cell change in multicellular organisms can be correlated with the rate of growth. Such data could probably be obtained readily from tissue cultures, for it is quite clear that the cells in a tissue culture behave, in general, as do the cells of bacteria in a culture. If allowed to remain in the same plasma, the cells differentiate after a time. If continually transferred to new plasma, they remain in the embryonic form, as is the case with bacteria.

Increase in size of the cells during the active growth period is accompanied by increased variability in size. This is clearly indicated by plotting frequency curves for the distribution of the cells according to size. Such curves show that during the logarithmic growth phase, the range and dispersion are greatly increased. When the cells again become small they are also more uniform in size, the range being greatly restricted (Henrici '26).

These measures of size have been made entirely by determining the length of the cells, since with the rod-shaped organisms this is the simplest and easiest measure to use. Because the cells increase more in length than they do in width, the embryonic forms, while they are actually somewhat more thick than the resting or mature forms, are relatively more slender. The form of the cells, as long as they vary only in plumpness or slenderness, can be readily determined by a measure which I have called the "area-length index." This is determined by measuring the area of the projected image of the cells with a planimiter and dividing this by the length squared. The more nearly the cells approach the spherical in form, the higher will be this area-length index. The more slender they become, the lower this value will be. Both the length and the area-length index of the cells have been determined for *Bacillus coli*. It was found that as the cells increase in size the area-length index decreases, that is, there is a negative correlation between these two values, indicating that the longer the cells become the more slender they become (Henrici '23). This seems to be generally true for all of the rod-shaped organisms.

In multicellular organisms the embryonic stage is followed by differentiation. Bacteria do not manifest in the resting phase any such variation in form and structure as is indicated by this term, but the cells in the resting phase are clearly different from those in the growth phase, not alone in size and form but also in internal structure, as manifested by less affinity for aniline dyes and by the development of new structures visible within the cell in the form of meta-

chromatic granules and spores. Spore formation always begins during the period when the growth curve shows a decrease in the rate of growth, and proceeds rapidly during the resting phase of the culture. That spore formation is dependent upon the rate of growth is proven by studying this phenomenon in cultures where the size of the seeding and the concentration of the nutrients have been varied. Both the rate of spore germination and of spore formation are changed by these factors. Spore formation is found to proceed more rapidly in those cultures which reach the resting phase earlier, that is, in the heavily seeded cultures and in the cultures with the least amount of nutrient (Henrici '24). Similarly, the metachromatic granules have been studied in a diphtheroid organism. Clark and Ruehl found that the diphtheria group differed from the others in that the young cells were small rather than large. This I have also found to be the case. The minimum size is attained shortly after the culture shows a change in growth rate from positive to negative acceleration, and then the cells begin to increase in size. More striking, however, are the changes in the metachromatic granules which rapidly decrease in number and almost completely disappear during the active growth period, and again slowly return. There is a degree of correlation, then, between the number of granules and the size of the cells. That these changes in cell structure are also dependent upon the rate of growth has again been demonstrated by varying the size of the seeding and the concentration of the nutrients. The culture heavily inoculated on a medium weak in nutrients showed only a slight change in the cells, which lasted for just a short time. After 15 hours, all cells had returned to the type which was used for seeding. With the same size seeding, but with more nutrient in the medium, more of the cells showed a change to the embryonic type, that is, they become small, deep-stained, and lost their granules, and a period of 24 hours was required for a return to the original form. While with the lighter seeding and full concentration of nutrients, the cells were still in the embryonic form at the end of 24 hours.

According to the theory which I am trying to establish, the senescent forms characteristic of the death phase are those forms which we have been in the custom of calling "involution forms," that is, forms which while not necessarily dead have been acted upon by the lethal factor, whatever it may be, which limits growth and which eventually leads to the death and dissolution of the cells. These are differentiated from the "normal" or resting cells by greater variation in form; by the production of lateral buds or branches, terminal swellings, or by a change of the entire cell to a spherical, or spiral, or other unusual type. Such variations in form are not readily studied quantitatively. I have tried first to measure these by the use of the area-length index previously mentioned. The organism of cholera is one of the bacteria which shows the widest variation in the form of the cells. Many authors have described transformations of this organism, normally comma-shaped cells, into straight rods and spheres or long spirals. By removing samples from the culture at regular intervals and measuring the forms of the cells, I have found that this organism undergoes a regular metamorphosis. During the initial lag phase the cells show no particular

change in form. They showed a wide variation from curved rods to small spheres with occasional budding and branching cells, that is, they remained involution forms, as they were in the parent culture. With the initiation of growth, however, the cells showed a marked transformation, they became large, straight, and thick, that is, they presented the appearance of rather plump bacilli. As soon as the rate of growth changed from positive to negative acceleration and the culture entered the resting phase, another transformation took place, the cells became slender and curved, that is, they assumed the normal vibrio form. This was followed by a further transformation during the time when the cells began to decrease in number. More and more small spherical cells, cells with swollen ends and with lateral buds and branches, appeared. By plotting frequency curves for the distribution of the cells according to their area-length index, it was found that during the active growth period they became more uniform in shape, as indicated by restricted range and a higher mode, while during the death phase the range became more extended, the curves became more irregular, showing a much greater variability in form (Henrici '25). Variability in form, then, is a characteristic of the death phase just as variability in size is a characteristic of the growth phase.

But the measure of form which I have used, the area-length index, is not entirely satisfactory because it does not serve to classify those types of cells which are most interesting and which have been the subject of the greatest amount of discussion; namely, cells showing asymmetry either in the occurrence of bulgings, or of constrictions, or of lateral projections. Such asymmetrical cells are the types which have been discussed most by the recent pleomorphists. They are found in cultures of practically all organisms but, as far as my observation goes, never until the culture has entered the death phase. They are particularly prone to develop in unusual media, or in strains of bacteria recently isolated from the animal body and not yet adapted thoroughly to saprophytic growth. In an attempt to determine something regarding the significance of these forms in relation to the rate of growth and death, I have studied a culture of *Bacillus coli* on several unusual media, such as have been used by previous authors for the production of these involution forms. The basic nutrient was 2 per cent peptone. This was divided into 5 lots, one being retained without any further treatment, and this was found to have a reaction of pH 7.4, another was made alkaline (pH 9.2), a third acid (pH 4.5). To the fourth 5 per cent sodium chloride was added, and the fifth received 3 per cent calcium chloride. Samples were removed at regular intervals after the first 24 hours and the rate of death and the degree of variation in the form of cells were determined. The cells were practically all alike at the end of 24 hours, no matter which medium was used; they became progressively more variable in form as the cultures became older, but this increased variability in form was different with the different cultures. In the alkaline medium there was little change; in the neutral medium there was slightly more variation in form, and this variation became progressively greater as we proceeded through the acid, the salt, and the calcium chloride media. The rate of death was measured in three ways; by agar plate counts, which gave the

decrease in cells as measured by their viability in a new medium; by microscopic counts, which gave the decrease of cells as measured by their complete autolysis and dissolution; and by the staining of the cells with Congo red, which I have previously shown is an indication of death and also of partial autolysis. In the death phase conditions are complex; while some cells are dying others are still growing, and for reasons which I have not time to explain in detail here, the microscopic counts are not a good measure for the death rate. It was found that the curve for percentage decrease of cells, as determined by the staining with Congo red, occupied in every case a position between the curves for percentage decrease as determined by plate counts and the curves for percentage decrease as determined by microscopic counts. But the distance between these curves varies with the different media. The rate of autolysis of the cells can be roughly measured by the distance between the curves for plate counts and the curves for stained cells, that is, by the time which elapses between the moment that the cells have lost their viability and the moment that autolysis has proceeded to such a degree that they will become stained by Congo red. This autolysis rate differed in the various cultures and was independent of the death rate. To measure the variability in the form of cells I have devised a procedure based upon the frequency distribution of the various types as determined by simple inspection. All of the cells found were divided into ten classes: normal rods, ovals, spheres, slender filaments, curved and spiral cells, cells constricted in the middle, club-shaped cells, cells with lateral buds and branches, cells with terminal buds, and cells with central bulgings. The percentage frequency of these types was determined in all of the samples in all stages of growth and from these the mean frequency for the entire series was computed. Each class was then given a value, the reciprocal of this mean frequency. Now returning to the individual cultures, the frequency of each class was multiplied by this reciprocal of its mean frequency, and the average of these values for the entire sample gives a measure of the variation in form. By this procedure the degree of variation of form is expressed according to the principle of index numbers. Those cultures which have the largest proportion of the most frequent types of cells will have a value less than one, and those cultures which have more than the average number of the less frequent types of cells have a value greater than one. By this procedure then, a quantitative measure is attained which, while not highly accurate, is probably sufficiently so to be significant, and this expresses the degree to which the form of the cells varies from the so-called normal form. These values were found to vary in rank order in the same way that the cultures were arranged according to their rate of autolysis. There is, therefore, a correlation between the variation in form and the rate of autolysis during the death phase, although no correlation could be established between the variation in form and the rate of death (Henrici '26). It would seem, then, that these bizarre forms characteristic of the death phase are dependent upon the autolysis of the cells. We may postulate that the normal cell form is maintained by a certain degree of rigidity in the cell wall. Anything which leads to softening of the cell wall will lead to a variation in form since the force of surface tension, where there

is no rigid membrane, will invariably tend to bring the cells to the spherical. If this softening of the cell wall involves only a small portion of the membrane, it will lead to lateral buds, or bulgings, or to terminal swellings, or to central bulgings, depending upon the location of the area first softened. If it involves the entire cell, it will lead first to the formation of oval, and finally spherical forms. That surface tension is an important factor is indicated by certain studies not yet completed, of the form of the cells in media containing soap. Here, in spite of the rapid death and autolysis of the cells, the normal form is nevertheless continued for a long time, the surface tension being much lower.

It may well be that the production of such structures as zygospores and conidia in higher fungi are also dependent upon the phase of growth and upon physical factors in the medium. But it is not necessary to assume, in order to explain the structures which are found in cultures of bacteria, that these structures are identical with or even analogous to such reproductive bodies found in higher fungi. In the phenomenon of cytomorphosis we have an explanation which will suffice for all the variations encountered. As organisms decrease in size the surface area in proportion to the volume of protoplasm becomes increasingly greater and such cells are therefore much more susceptible to the action of physical forces, particularly surface tension, than are larger organisms. Thompson ('17) has pointed out the serious error involved in interpreting similarity of form in minute organisms as an evidence either of phylogenetic relationship or of physiologic function. Because of this vastly greater influence of external factors in minute cells, before we can safely assume that the bizarre cell types found in certain cultures of bacteria are special reproductive bodies, parts of a complex life cycle, we must first determine beyond any question of doubt, by continuous observation, that each of these is reproductive in the sense that it will again give rise to normal cells, and in the case of those structures interpreted as sexual spores, that they are actually formed by the conjugation of two cells. Such continuous and complete observation has been conspicuously lacking.

My investigations have clearly pointed out one serious source of error in previous work on mutations and variations in bacteria. Since there is a separate cell-form for each phase of growth, before we can claim that an organism has undergone a permanent variation in form, we must clearly determine the phase of growth in each culture studied. Since the growth curves can be altered by such slight factors as variations in the number of inoculated cells or in the amount of nutrient in the media, you will see that when two cultures growing on different media or inoculated from different cultures are compared, one may still be in the embryonic form while the other may have entered the resting phase. With the diphtheroid organism which I have mentioned, the one would present small coccoid cells, deep staining, and without granules, looking much like streptococci, while the other would present long rods, irregularly distributed, and containing striking metachromatic granules. With the cholera vibrio one culture might present plump rods, while another, having entered the resting phase, would

show slender curved cells, and still a third which had grown rapidly and proceeded to the death phase, would present many coccoid forms.

But I feel that this phenomenon of cytomorphosis in bacteria has a bearing upon general biology even more important than its explanation of pleomorphism in bacteria, for if we grant that the cell changes in a culture of microorganisms—correlated with the phases of growth—are of the same kind as the cell changes which occur in a multicellular organism, we imply that there is no fundamental difference in the laws which govern the development of a population of free single cells and the development of an organized multicellular individual, and this further implies that the differentiation of the cells of the multicellular organism is not necessarily due to the multicellular organization of that individual. That this is the case is apparent from studies of tissue cultures where isolated cells grown in plasma can be caused to differentiate or dedifferentiate according to the volume, or frequency of renewal of the plasma.

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THE PRESENT STATUS OF BACTERIAL TAXONOMY AND NOMENCLATURE[1]

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It is in some respects unfortunate that this, the first International Congress in sixteen years for those interested in the plant sciences, should be incompetent to enact legislation, and that at this time no modifications of the nomenclatural codes are possible by international agreement. I believe that the bacteriologists could agree on a few at least of the modifications and amendments that would be helpful. On the other hand, it has been many years since it has been possible for adequate international discussion to occur, and it is not improbable that the four years which will probably elapse before another congress of this type convenes may be utilized profitably in formulation of definite proposals and in preliminary discussions.

Certain of our problems in bacteriological nomenclature differ somewhat from those of most other groups of plants with the possible exception of the fungi. I wish to recount certain of our special difficulties and to make certain specific recommendations relative to them.

The first difficulty that has caused some confusion is the fact that for many years, in fact until the time of the work of Cohn, microorganisms now included with the bacteria were included partly in the plant and partly in the animal kingdoms. The species described by Mueller (1773 and 1786) were placed by him among the animalcules, principally protozoa. On the other hand, the genus *Polyangium*, now quite definitely included among the bacteria, was described as a fungus in 1795. The bacterial genera *Serratia* (Bizio 1823), *Sphaerotilus* (Kützing 1833), *Beggiatoa* (Trevisan 1842), *Sarcina* (Goodsir 1842), *Leptothrix* (1843), and the series of genera named by Leidy of Philadelphia (1849–52) were all definitely described as plants before Cohn (1854) recognized the plant position of the bacterial group as a whole, and Naegeli (1857) definitely united genera previously described from both plant and animal kingdoms into the group Schizomycetes. Up to this time (1857) a considerable number of protozoan genera had been described which contained one or more species of bacteria as we now know them, such as *Monas*, *Vibrio*, *Bacterium*, *Spirillum*, *Spirochaeta*, etc. These genera have been distributed between plants and animals in our present systems of taxonomy. It should be clearly recognized that this dual origin of bacterial names introduces certain complications. The zoologists in

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their code indicate quite clearly the treatment to be accorded to names of organisms transferred from the plant to the animal kingdom. This has not been satisfactorily accomplished for forms transferred from the animal to the plant kingdom by the botanical code. I would therefore offer the following specific resolutions for your consideration or for reference to a suitable committee:

Resolution No. 1

Be it resolved by the Bacteriological Section of the International Congress of Plant Sciences, convened at Ithaca, N. Y., that a suitably appointed and competent international committee be asked to draft an amendment to the International Botanical Code which will indicate clearly the nomenclatural status and disposition of names of organisms transferred from the animal kingdom to the plant kingdom, such amendment to be presented to the next International Botanical Congress.

One of the real difficulties with the use of the International Code in the naming of bacteria by bacteriologists has been for most of them its relative inaccessibility. It has been printed in certain botanical publications and in proceedings relatively unknown and unused by bacteriologists in general. Unquestionably its very existence has been unknown to many bacteriologists who have been active in the description of species and genera. I would therefore suggest that provision should be made immediately, following each competent international congress, to provide for the adequate distribution of copies of the International Botanical Code to bacteriologists.

Apparently there is a fairly general agreement among bacteriologists and probably among mycologists that the insertion of the provision for Latin diagnoses of all species of plants published since 1908 is not practicable in its application to bacteriology. This article reads in part:

Art. 36. On and after January 1, 1908, the publication of new groups of living plants will be valid only when they are accompanied by a Latin diagnosis.

We as bacteriologists have no quarrel with those specialists in the taxonomy of any group who really desire to see this rule enforced in their own group. It is a dead letter in bacteriology. This has been recognized by the Committee on Bacterial Classification of the Society of American Bacteriologists who have recommended that such provision should not apply to the bacteria. To put the matter concretely I would suggest the following:

Resolution No. 2

Be it resolved by the Bacteriological Section of the International Congress of Plant Sciences, Ithaca, that the International Interim Committee, or some other competent committee, be requested to draft an amendment to the International Botanical Code which will clearly and definitely exclude bacteriological nomenclature from the requirement of Latin diagnosis of new forms described, such amendment to be presented to the next International Botanical Congress.

The International Botanical Code specifies clearly for most groups of plants the point of departure, or the true beginning of nomenclature in the group. For most groups of plants this has been fixed with the first edition of Linné's "Species Plantarum" of 1753. For certain groups, usually those not included by Linné, the date of some early monographic treatment is specified. For example, the

date of departure for the mosses was fixed as the publication of Hedwig's "Species muscorum" in 1801. A footnote to Article 19 (which fixes these dates) of the Brussels code states that the designation of the points of departure for some four groups of plants was reserved to the Congress of London to be held in 1915. One of the groups thus specified was Schizomycetes. There is, therefore, no fixed or authoritative starting point for the nomenclature of the bacteria internationally accepted at the present time. While not the only factor which may be taken into consideration in the determination of validity of bacterial names, priority is of major importance. It is essential therefore that a starting point be fixed. If that point is to fixed at any time subsequent to 1753, it seems that it should be the time of publication of some treatise on bacteria which possesses the following characteristics:

(1) It should subscribe and adhere in general to the established usages of binomial nomenclature.

(2) It should be a relatively complete survey of the species of the group.

Several points of departure for bacteriological nomenclature have been suggested. At the Brussels Congress, Vuillemin made two suggestions. The first was that 1753 be accepted. This has the advantage of antedating all descriptions of bacteria. It brings the bacteria into line with most other plant groups, and places the date of departure near that (1758) recognized by the zoologists in their International Code. The first genera and species of bacteria apparently were named by Mueller in 1773, and others in 1786. These publications were primarily treatises on protozoa and other animalcules. Neither account can in any sense be regarded as a monographic treatment of the group of bacteria. There is no apparent advantage to be gained by the adoption of either of these dates rather than 1753. The brilliant treatise of Ehrenberg (1828) on the Infusoria includes descriptions of certain bacterial species and genera. As a point of departure for bacteriological nomenclature it has the same defect as those of Mueller, it is a zoological treatise. It does not include species and genera of bacteria described previously as plants. Apparently no other treatise or date is available thereafter until 1872, when Cohn began the publication of his classic series of papers on the bacteria as plants. In many respects these would constitute a satisfactory starting point. This date (1872) was proposed at Brussels by Klebahn and approved by Engler. However, a careful study of Cohn's papers will show that his knowledge of the bacterial forms previously described was not complete. The acceptance of this date would mean discarding several well established generic names, and, it would seem, without compensating advantages. The suggestion was also made formally by a Committee of the Society of American Bacteriologists (1918) that the date of departure be fixed by the third edition of Zopf's "Die Spaltpilze" (1885). This likewise is an attractive suggestion, but attempts to put it into effect have encountered the following difficulties:

(a) The author did not adhere strictly to the established principles of binomial nomenclature.

(b) The treatise is not sufficiently inclusive and monographic.

(c) It discards much valuable work done previously by other writers.

Among the other dates which might possibly be regarded as available are the following:

(a) The treatment of Schizomycetaceae by De Toni and Trevisan in Saccardo's Sylloge Fungorum. (Vol. 8, 1889).

(b) Lehmann and Neuman's text of 1896.

(c) Migula's System of 1900.

In 1910 Vuillemin made the statement that if a date subsequent to 1753 were adopted as a point of departure, it might well be 1910. He suggested that a list of generic names be more or less arbitrarily decided upon, approved by the next Congress, and bacterial nomenclature date from this. In 1913 he published a proposed list for recommendation to the London Congress which was to have met in 1915. An examination will show its defects at once, it will not stand critical analysis.

On the whole, I believe that there is a growing sentiment that little will be gained and much will be lost by fixing a date of departure later than that of the recognized beginnings of bacteriological nomenclature. I would suggest therefore a third resolution:

Resolution No. 3.

Be it resolved by the Bacteriological Section of the International Congress of Plant Sciences, Ithaca, that the International Interim Committee, or some other competent committee be informed that in the opinion of this Section there is no sufficient reason for the establishment of a point of departure for the nomenclature of the bacteria subsequent to 1753, and that this Section suggests that this date be incorporated into the International Botanical Code.

There is also a growing appreciation of the type concept among botanists in general, and in particular, I believe, among bacteriologists, and of the value of this concept in stabilizing nomenclature. This is recognized in a recommendation in the International Code which reads as follows:

"When names of new groups are published the author should designate the subdivisions which he considered as the type for the nomenclature of the group; he should indicate the generic type in a family, the species type in a genus, the subspecies or variety type in a species. This precaution will prevent difficulties in nomenclature if it should become necessary later to dissociate such groups."

Article 45 also states that when a genus is divided the original generic name must be reserved for the type group. The zoologists are much more explicit in their code, and insist that family names shall be formed from the generic name of the type genus. Beyond reasonable doubt, we in bacteriology would be aided by greater emphasis upon the type concept if it can be satisfactorily employed. There are certain difficulties, however, which must be recognized.

It seems quite hopeless to find among all the definitions that have been proposed for the concepts species and genus any that will give natural delimitations of such groups. The limits must be elastic, and adjustable to the concepts of different individuals. To me the only definition of a species of plant that is at all satisfactory is somewhat as follows: "A species of plant is a particular plant individual, usually a particular specimen, together with such others as resemble it sufficiently to be included in the same natural group." Similarly,

"A genus is a particular type species, together with such other species as resemble it sufficiently closely to be included in the same natural group."

The fundamental feature of the type concept, of course, is the type specimen or specimens. A suggested American amendment to the code reads, "The nomenclatural type of a species is the specimen or the most important of the specimens upon which its original published description was based."

It is evident that this definition of type species is quite ambiguous when used for bacteria. Just what would be meant by the term "specimen" as applied to such forms? Presumably, for organisms described from *pure cultures* it would mean *the* pure culture upon which the original description was based. In bacteriology our descriptions and identifications are in the great majority of cases of *living* bacteria. The higher plant may be dried, the insect mounted, the fish preserved, the mammal may be skinned and the skeleton preserved. Differentiation among these forms has been almost entirely based upon morphology. We cannot identify bacteria from bits of pure culture dried upon squares of mica such as are found in some of the older fungous exsiccati. In several places in Europe and in American so-called type culture collections are maintained, the organisms being continually grown in pure culture. Presumably the cultures preserved are those whose histories are known. However, experience has taught us that not always are we able to withdraw the same kind of organism as was deposited even when we are quite positive we are not dealing with mutants.

Probably the closest approach to a real type specimen that a bacteriologist may hope to secure is a culture which is a transfer from the original strain, and which upon study is found to agree with the original description. In the great majority of cases no such cultures or transfers of described species are in existence. The type is missing. It is apparent that we must depend almost entirely upon careful, accurate, and complete descriptions, in most cases.

Much more important in stabilizing our bacteriological nomenclature is to fix definitely the type species of each genus. This does not appear to be a serious difficulty, apparently such types can be readily fixed by name, at least in most cases. I would, therefore, sugest some such statement as follows:

Resolution No. 4.

Be it resolved by the Bacteriological Section of the International Congress of Plant Sciences, Ithaca, that the International Interim Committee, or some other competent committee be informed of the belief of this Section that greater emphasis in the International Code should be placed upon the so-called type concept, with the request that no provision may be incorporated which will make necessary the designation of a "type or standard specimen," in the ordinary botanical sense, for the bacteria.

There is in bacteriology, apparently, an effort on the part of some individuals to prescribe or limit in some arbitrary fashion the list of kinds of characters which may be used in the delimitation of bacterial species and of bacterial genera. Recently the insistence upon morphology as a sole basis of species differentiation has disappeared. Personally, I feel that the establishment of any restrictions as to the kinds of characters which may be utilized in the differentiation of microorganisms would be arbitrary, unauthorized, and unfortunate. It would

seem that any code proposed, adopted, or amended should be carefully edited to make sure that there is no implication that morphology shall constitute the sole criterion for the differentiation of groups of bacteria of any rank.

Finally, from the standpoint of stability in our bacteriological nomenclature, it is highly desirable that some international action be made possible. It would be most unfortunate if bacteriologists should insist upon a separate code for the particular group of plants with which they deal. It is far better to follow as far as is possible the International Botanical Code. But we have certain special needs. I do not believe there would be any serious objection to the inclusion in the Code of certain provisions of particular use to the bacteriologist. But we must know what we want. I would therefore suggest an additional resolution.

Resolution No. 5.

Be it resolved by the Bacteriological Section of the International Congress of Plant Sciences, Ithaca, that the International Interim Committee, or other competent Committee designated by them, be requested to select a special international and representative committee of bacteriologists, which committee will have as its particular function the coördination of the particular nomenclatural interests of bacteriologists with those of other botanists, such committee to report its findings and recommendations through the regular channels in preparation for the next International Botanical Congress.

So much for nomenclature and resolutions: I wish now to say a few words only on the subject of classification.

Bacteriology is a science which from the beginning has had its practical and economic applications. A larger proportion of the known species of bacteria are of economic importance probably than in any other plant group. Uniformity and stability in nomenclature are therefore even more desirable in this group than in most others; the scientific names are more widely used by those not particularly interested in problems of taxonomy.

What are the difficulties in the way of satisfactory classification and differentiation of the bacteria? Why do we not have even yet suitable and acceptable classifications, keys, and descriptions entirely comparable to those which we have for the flowering plants?

It must first be emphasized that, in general, studies of bacteria have been primarily of species or groups of known, fancied, or suspected economic importance. To find an organism and demonstrate its economic significance has proved far more interesting and enticing than to prepare careful and accurate descriptions. It is probably quite impossible ever to identify any considerable proportion of the bacterial species which have been named. Apparently there is no journal or publication which has even regularly listed these names as they have appeared.

Then, too, we have a major difficulty in that the criteria which have been adopted by the workers in different economic groups show great variation in spite of standard charts and other aids. The soil bacteriologists will make certain studies of morphology, culture relations, and physiology which seem to him important; the plant pathologist will isolate the same organism and describe it in a different set of terms; and the sanitary bacteriologist will try it on his

special differential media. Until greater care is used in the selection of criteria for differentiation of bacteria we shall have difficulty both in nomenclature and in classification.

Nor do I believe that there is any advantage to be gained by any premature insistence upon standardization. Quite wisely, I believe, the Society of American Bacteriologists has made certain recommendations for consideration, but has not attempted to legislate. Possibly the time may come when we may have an accepted check list of recognized species of bacteria, a list similar to that maintained by our ornithological friends, but certainly it is a long way in the future.

This is all by way of emphasizing the great present necessity of monographing the various groups. Comparatively little of this has been done. Even a preliminary or fairly satisfactory classification will not be possible until we have adequate treatment of the various groups in monographic form by writers who have knowledge both of the organisms and of the fundamental principles of nomenclature.

ENERGY UTILIZATION AND CARBON ASSIMILATION OF AUTOTROPHIC BACTERIA[1]

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Autotrophic bacteria obtain their energy chemosynthetically, that is, from the oxidation of simple inorganic substances; the carbon necessary for the synthesis of the cell constituents is obtained from carbon dioxide of the atmosphere or from carbonates in solution. The heterotrophic bacteria obtain their energy and carbon from complex organic materials, either by oxidation or by intramolecular structural changes. Notwithstanding the fact that more than forty years have passed since Winogradsky first studied the physiology of sulphur bacteria, and that there have since been described a number of organisms which are capable of living autotrophically, the subject of carbon and energy utilization by autotrophic organisms still calls forth certain vague generalizations and hazy conceptions indicating that a clear idea of the processes involved has not as yet penetrated into the texts. To illustrate this, it is sufficient to cite a paper of a prominent American bacteriologist (Kendall '24), who states, "The absence of chlorophyll, or other photodynamic agent, makes the bacteria dependent upon preformed or organic food. This suggests that they are some distance removed phylogenetically from the probably primordial life, because the latter must have been independent of all substances of organic origin." This primordial life is represented at present in millions of organisms in every particle of soil, in springs, in sewage disposal plants and, in general, all around us.

Various recent investigations seem to point to the fact that all micro-organisms, aerobic and even anaerobic forms, require various amounts of carbon dioxide. Nothing definite is known concerning just what use is made of this gas in the metabolism of the organisms. However, in the case of the autotrophic bacteria, we know definitely that this gas is utilized as a source of carbon. In the absence of all traces of organic matter, but in the presence of carbon dioxide and traces of minerals, these bacteria will synthesize organic matter, given only a proper source of energy, such as ammonia, nitrite, sulphur, hydrogen, methane, etc.

According to Winogradsky ('22), obligate autotrophic bacteria, or anorgodydants, are characterized by a series of physiological properties, which may be summarized as follows: (1) Their development in nature takes place only in strongly elective, almost pure mineral media, which contain specific oxidizable inorganic substances. (2) Their existence is connected with the presence of

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these substances which undergo oxidation as a result of the life activities of the organisms. (3) This oxidation process supplies their only source of energy. (4) The organisms do not need any organic nutrients for development or for energy. (5) They are almost incapable of decomposing organic matter and may be even checked in their development by its presence. (6) They use, as an exclusive source of carbon, carbon dioxide, which is assimilated chemosynthetically.

These original conceptions of Winogradsky have to be modified in two ways: (1) The presence of organic matter may not prove injurious to the activities of the autotrophic bacteria. As a matter of fact, the presence of small quantities of certain organic substances may even be stimulating to some species. Further, the existence of these organisms in the soil, where they carry on their life processes, takes place in the presence of soluble organic substances. (2) Only a few of the autotrophic bacteria are obligate, some are facultatively autotrophic. The latter, as in the case of some sulphur, hydrogen, and methane bacteria, can exist both autotrophically and heterotrophically.

The autotrophic bacteria may be classified on the basis of the elements and compounds from which they are able to obtain energy:

(1) *Nitrogen and its compounds.* We do not know as yet whether the nitrogen-fixing bacteria are capable of utilizing any energy which may be liberated in the process of transformation of the elementary nitrogen into the first compound of nitrogen. Only two groups of organisms which are capable of obtaining energy from the oxidation of nitrogen compounds are known: (a) those that oxidize ammonia to nitrites, and (b) those that oxidize nitrites to nitrates; both of these are usually classified together under the term "nitrifying" bacteria. (Winogradsky '90, '91.)

(2) *Sulphur and its compounds.* The compounds include the reduced and partially oxidized compounds of sulphur, H_2S, sulphides, thiosulphates and, perhaps, sulphites and hyposulphites. The organisms obtaining their energy from the oxidation of sulphur and its compounds, especially the sulphides, belong to morphologically different groups.

(3) *Selenium and its compounds.* Our information as to the energy utilized from the oxidation of these substances is not yet definite.

(4) *Iron compounds.* The most important of these is ferrous carbonate. As shown elsewhere, not all organisms which are capable of precepitating ferric hydroxide are autotrophic bacteria.

(5) *Hydrogen and simple hydrocarbons.*

(6) *Carbon and some of its compounds.* These include carbon monoxide, methane, higher hydrocarbons, and alcohol. It is not definitely known whether any organisms are capable of obtaining energy from the oxidation of elementary carbon.

Of these various groups of bacteria, only three forms are so far known to be obligately autotrophic, namely, *Nitrosomonas*, *Nitrobacter*, and *Thiobacillus thiooxidans*. These are the only organisms which cannot obtain their energy from complex organic substances, although, in the case of the last mentioned form, at least, the addition of a small amount of glucose or other soluble car-

bohydrate is not only uninjurious but may exert a certain stimulating effect. It is as yet unknown what the nature of this stimulus may be, since the ratio of sulphur oxidized (energy source) to the amount of carbon assimilated from the CO_2 of the atmosphere (synthesis of cell substance) remains constant, in the absence and presence of the carbohydrate. The formation of nitrites from ammonia by thiosulphate bacteria (Klein '23) and the decomposition of celluloses by nitrate-forming bacteria (Sack '24, '25), observed by some investigators, are still phenomena to be investigated further.

The autotrophic bacteria are the only microscopic organisms, with the exception of the algae (obtaining their energy photosynthetically), which are capable of doing work in the true thermodynamic sense. Accurate information can be obtained in the case of these organisms regarding the amount of chemical energy required to build up complex organic substances from minerals and carbon dioxide. The simple composition of the nutrients helps to differentiate structural and functional energy required in metabolism; it also throws light on the influence of concentration of nutrients, neutralization of waste products, and amount of total available energy utilized by the organisms in connection with carbon assimilation.

The processes of energy utilization in the case of nitrite and nitrate forming bacteria may be represented as follows:

$$2\ NH_3\ (acq) + 3\ O_2 = 2\ HNO_2 + 2\ H_2O + 156.8\ Cal.$$
$$2\ HNO_2 + O_2 = 2\ HNO_3 + 37.6\ Cal.$$

The synthesis of cell substance may take place in a manner similar to photosynthesis, by one of the following reactions:

$$(1)\quad 2\ NH_3 + 2\ O_2 = 2\ HNO_2 + 2\ H_2$$
$$CC_2 + 2\ H_2 = HCOH + H_2O$$
$$(2)\quad CO_2 + H_2O = HCOH + O_2$$

The energy obtained in the reactions of oxidation is utilized for the assimilation of CO_2, Meyerhof ('16- '17) having shown that the N:C ratio, or the amount of nitrogen (in the form of nitrogenous substance) oxidized to the carbon (in the form of CO_2) assimilated is 35 in the case of ammonia oxidation and 135 in the case of the nitrite oxidation. The first reaction thus liberates (78.4 Cal for 1 mol NH_3 oxidized) four times as much energy as the second reaction (18.8 Cal per 1 mol HNO_2 oxidized), but only one-fourth as much substrate is transformed (35/135), indicating that the actual amount of energy utilized for the carbon assimilation and synthesis of cell substance is about the same in both reactions. One can easily calculate that a little over 5 per cent of the energy liberated in the oxidation processes is utilized for doing actual work in the synthesis of protoplasm from CO_2, inorganic nitrogen, and minerals.

A similar phenomenon is observed in the chemosynthetic utilization of energy from sulphur compounds. The sulphur bacteria do not constitute a uniform group of microorganisms as in the case of the nitrifying organisms, either morphologically or physiologically. Morphologically they are found among the

Desmobacteriaceae (Thiobacteriales) and among the Bacteriaceae (Eubacteriales). Physiologically they may oxidize hydrogen sulphide and other sulphides, elementary sulphur, thiosulphate, and they may act either at an acid or at an alkaline reaction. Some are obligate autotrophic forms and some are facultative. The bacteria which are found in normal, fertile soils, or those that become active in the soil, when introduced, are limited chiefly to the genus *Thiobacillus* among the Bacteriaceae.

All microorganisms require minute quantities of sulphur for the synthesis of protoplasm. Various bacteria and even some fungi seem to be capable of oxidizing small amounts of sulphur. But some bacteria work over much larger quantities of sulphur than would be necessary for their body structure, since they utilize the sulphur or its compounds as a source of energy. The sulphur is to the sulphur bacteria, as the ammonium sulphate is to the *Nitrosomonas* and *Nitrosococcus*, the nitrous acid and nitrite to *Nitrobacter*, and the carbon compounds to the heterotrophic bacteria.

The sulphur bacteria, or those bacteria which are capable of obtaining the energy necessary for their growth from the oxidation of sulphur or its compounds, should be distinguished from other bacteria taking part in the sulphur cycle such as those liberating H_2S in the hydrolysis of proteins or in the reduction of sulphates.

The oxidation of hydrogen sulphide takes place as follows:

$$2\ H_2S + O_2 = 2\ H_2O + S_2 + 122.2\ \text{Cal}\ \text{(Winogradsky '83–'87)}$$

The reaction $H_2S = H_2 + S$ is endothermic, but the simultaneous oxidation of hydrogen makes the process exothermic.

The oxidation of sulphur itself takes place as follows:

$$S_2 + 3\ O_2 + 2\ H_2O = 2\ H_2SO_4 + 284.4\ \text{Cal}\ \text{(Winogradsky '22).}$$

In the oxidation of elementary sulphur and assimilation of carbon from CO_2 by *Thiobacillus thiooxidans*, the ratio of $S:C$ was found to be 32 (Waksman and Starkey '23, Starkey '25). About 6.5 per cent of the heat liberated in the oxidation process is utilized for the synthesis of cell substance. The *T. thiooxidans* is the only organism known which oxidizes sulphide directly to sulphate, without the intermediary formation of sulphur. The same is true of the oxidation of thiosulphate.

Various reactions have been suggested to explain the mechanism of oxidation of thiosulphate, due to the fact that different organisms show distinct differences in the process. The *T. thioparus* studied by Nathanson ('02) and Beijerinck ('04), also found abundantly in nature (in the air, soil, etc.), oxidizes the thiosulphate with the precipitation of elementary sulphur. Nathanson believed that the following reaction takes place:

$$3\ Na_2S_2O_3 + 5\ O = 2\ Na_2SO_4 + Na_2S_4O_6$$

The tetrathionate interacts with some of the remaining thiosulphate chemically liberating free sulphur. Beijerinck suggested that the sulphur is liberated directly from the thiosulphate:

$$Na_2S_2O_3 + O = Na_2SO_4 + S$$

Trautwein ('21–'24) isolated an organism which was capable of oxidizing thiosulphate, under anaerobic conditions, using nitrate as a source of oxygen, without the precipitation of sulphur but also without the formation of acid in the medium:

$$4\ Na_2S_2O_3+6\ O_2=4\ Na_2S_2O_6$$
$$3\ Na_2S_2O_3+5\ O=Na_2S_4O_6+2\ Na_2SO_4$$

The oxidation of thiosulphate by *T. thiooxidans* takes place without the precipitation of sulphur but with an active increase in acidity.

$$Na_2S_2O_3+2\ O_2+H_2O=Na_2SO_4+H_2SO_4$$

Of the 3 Thiobacilli isolated, the *T. thioparus* and *T. denitrificans* are facultative autotrophs, while the *T. thiooxidans* is an obligate autotroph. Of the thread-forming bacteria, *Beggiatoa* and *Thiothrix* are autotrophic while the purple bacteria are not (Keil '12). Very little is known of the autotrophy of the various other sulphur bacteria which accumulate sulphur within their cells.

Very little is known also of the energy utilization of the organisms capable of oxidizing selenium and selenites. The iron bacteria form an interesting group of organisms. The strictly iron bacteria, or those organisms that are capable of oxidizing iron carbonate ($FeCO_3$) into ferric hydroxide, whereby the energy obtained is used for the chemosynthetic assimilation of carbon, should be distinguished from those organisms that can absorb or accumulate iron, when living in iron-containing media. The accumulation of iron by the latter is not physiological, but mechanical, while in the case of the former the iron compound is formed by a chemical process and not merely accumulated (Winogradsky '88).

According to Lieske ('11 '19), *Spirophyllum ferrugineum* can grow in inorganic media free from organic matter and oxidize ferrous carbonate to ferric hydroxide. The energy thus liberated is utilized by the organism for the chemosynthetic assimilation of CO_2.

$$2\ FeCO_3+3\ H_2O+O=Fe_2(OH)_6+2\ CO_2+29.8\ \text{Cal}$$

The amount of energy liberated is very small (15 Cal per 1 mol ferrous carbonate or 0.12 Cal per 1 gram) in comparison with that liberated in the oxidation of the inorganic nitrogen and sulphur compounds. Large quantities of iron have to be oxidized by the organism to liberate sufficient energy necessary for the carbon assimilation, which results in the accumulation of considerable iron hydrate in the bacterial cells. Actually, in the assimilation of one part of carbon, about 750 parts of iron hydrate will be formed.

Harder ('19) very properly divided the iron bacteria into 3 groups, on the basis of their physiological activities, as follows:

(1) Those that precipitate ferric hydroxide from solutions of ferrous bicarbonate and use the carbon dioxide liberated and the energy produced during oxidation for their life activities (autotrophic).

(2) Those which do not require ferrous bicarbonate for their life processes but which deposit ferric hydroxide when either inorganic or organic iron salts are present (heterotrophic).

(3) Those which attack organic iron salts, using the organic acid radical as a nutrient, precipitating ferric hydroxide or basic ferric salts, which are gradually changed to ferric hydroxide. Inorganic iron salts, however, are not utilized as sources of energy and this group is also heterotrophic.

Hydrogen can be utilized as a source of energy by a number of bacteria, including non-spore-forming and spore-forming organisms, all of which are facultatively autotrophic; in other words, such organisms are capable of using this source of energy when it is present, otherwise they can thrive just as well on organic materials.

The oxidation of hydrogen is accompanied by the liberation of large quantites of heat:

$$H_2+O=H_2O+68.4 \text{ Cal}$$

One gram of hydrogen liberates 8 times as much heat of combustion (34.2 Cal) as 1 gram of starch (4.1 Cal). The mechanism of hydrogen oxidation may depend on the nature of the organism. Kaserer ('05) suggested that formaldehyde is formed as the first step in the synthesis of cell substance, that is,

$$H_2CO_3+2\ H_2=CHOH+2\ H_2O$$
$$CHOH+O_2=H_2CO_3$$

For every volume of CO_2 assimilated, 2 volumes of hydrogen are oxidized and one-half volume of oxygen is utilized. However, these were not results obtained by actual experimentation, but were largely based upon assumptions. Lebedeff ('10) could not demonstrate any formaldehyde as an intermediary product in the assimilation of CO_2. The ratio of hydrogen oxidized to CO_2 assimilated was found to depend on the age of the culture, being wider in older than in younger cultures. The energy process was believed to be independent of the process of carbon assimilation.

The gas exchange in the autotrophic assimilation of carbon by these bacteria was found similar to that of green plants. In the presence of organic matter, the organism oxidizes considerably less hydrogen than in the absence of organic matter.

The optimum reaction for the growth of the hydrogen organism was found by Ruhland ('24) to be pH 6.8–8.7. On the acid side of this optimum, growth diminishes due to the disappearance of the HCO_3 ion and to the direct action of the hydrogen ions upon the organism. On the alkaline side of the optimum growth diminishes, due to an increasing diminution of available iron. Different CO_2 pressures influence the growth of the organism by influencing the reaction. The hydrogen-oxygen ratio is usually greater than 2 to 1, reaching 2.78 due to the formation of free oxygen in the reduction of CO_2.

The utilization of energy in the oxidation of simple carbon compounds may be looked upon as a kind of connecting link between the purely autotrophic and the heterotrophic utilization of energy. While the oxidation of methane and carbon monoxide may be looked upon as purely autotrophic, the oxidation of acetic acid and especially of hydrocarbons may be classified with the heterotrophic processes, since some of the intermediary or final products of oxidation

may be utilized by the organisms for the synthesis of cell substance rather than for the production of CO_2.

In the oxidation of methane by bacteria, considerable heat is liberated:

$$CH_4 + 2\,O_2 = CO_2 + 2\,H_2O + 218 \text{ Cal}$$

The bacteria capable of utilizing this energy are facultatively autotrophic; they develop in an atmosphere rich in methane, with an optimum concentration of oxygen at 2 per cent. The methane cannot be replaced by hydrogen or carbon monoxide but can be replaced by various organic compounds, such as, alcohols, salts of organic acids, carbohydrates, etc.

Similar considerations hold true for the oxidation of carbon monoxide.

$$CO + O = CO_2 + 74 \text{ Cal}$$

The acetic acid bacteria are usually divided (Janke '21), on the basis of carbon utilization, into 2 groups: (1) *haplotrophic*, or those which obtain their energy from the oxidation of alcohol, and (2) *symplotrophic*, or those which require organic compounds. The former are the only ones that need be considered here.

$$CH_3CH_2OH + O_2 = CH_3COOH + H_2O + 112 \text{ Cal}$$

The energy liberated by these bacteria is utilized for the synthesis of microbial protoplasm. No other organic substances are required either as sources of energy or carbon. However, in view of the fact that in the above reaction acetaldehyde forms an intermediary product and this can be used directly by the bacteria for the synthesis of cell substance, without any actual consumption of CO_2 for this purpose, it is questionable whether we should include even these organisms among the autotrophic forms.

$$CH_3 \cdot CH_2 \cdot OH \rightarrow CH_3 \cdot COH \begin{cases} \rightarrow CH_3 \cdot CH_2 \cdot OH \\ \rightarrow CH_3 \cdot COOH \end{cases}$$

Energy is liberated in the oxidation of the ethyl alcohol to acetaldehyde, but only a third of that energy is liberated in the oxidation-reduction of the acetaldehyde (Neuberg '15).

The nature of energy utilization by bacteria capable of oxidizing hydrocarbons and amorphous carbon still remains to be studied.

Summary

The autotrophic bacteria, or those bacteria which are capable of obtaining their energy from the oxidation of inorganic substances and their carbon from the carbon dioxide of the atmosphere, do not form, either morphologically or physiologically, a closely related group of organisms. Morphologically they are found among the thread-forming bacteria and the Eubacteriales, the latter including cocci, non-spore-forming and spore-forming bacteria. Physiologically, they range from obligate autotrophs to facultative autotrophs, finally to such organisms as the acetic acid bacteria, which may even be conveniently classified with the heterotrophic forms.

The autotrophic bacteria may be classified into 6 groups on the basis of the specific element (or its compounds) which they are capable of oxidizing, util-

izing the energy liberated for the chemosynthetic assimilation of carbon: (1) Nitrogen and the inorganic compounds of this element, (2) sulphur and its compounds, (3) selenium and its compounds, (4) inorganic iron compounds, (5) hydrogen and its compounds, (6) carbon and simple compounds of carbon. Only three obligate autotrophic bacteria are known, namely, *Nitrosomonas*, *Nitrobacter*, and *Thiobacillus thiooxidans*. These organisms cannot obtain their energy or their carbon from organic substances, but require the specific inorganic element or compound as a source of energy and carbon dioxide or carbonates as a source of carbon. All the other autotrophic bacteria are facultative, capable of obtaining their energy and their carbon also from complex organic materials.

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DIE CHARAKTERISTIK DER BAKTERIOLOGISCHEN PROZESSE IM SCHWARZEN UND ASOWSCHEN MEEREN[1]

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Heute zweifelt man nicht mehr daran, dass den Mikroorganismen im Leben der Ozeane und Meere dieselbe Rolle gehört, wie in verschiedenen von ihnen bewohnten Schichten des Bodens.

Die Erforschung der Meere in Bezug auf die dort stattfindenden Prozesse, welche dem Merre eine besondere Eigenschaft verleihen, nahm ihren Anfang später als die Bodenuntersuchungen. Den Hauptanstoss zu diesen Forschungen gab, möchte ich sagen, die Entdeckung des Schwefelwasserstoffes in den Tiefen des Schwarzen Meeres und ferner die Hypothese Brandts über die Rolle der denitrifizierenden Bakterien in der Ökonomie der Meeresbecken.

Die Erforschung der Meerrestiefen ist mit grösseren Schwierigkeiten verknüpft, als die Erforschung der Prozesse in der Erdschicht und daher ist es erklärlich, dass die bakteriologischen Prozesse in den Meeren weniger Beobachter fanden, als solche im Boden. Die letzten Untersuchungen wurden durch den engen Zusammenhang zwischen den bakteriologischen Prozessen im Boden und der Kultur der Nutzpflanzen unterstützt. Dagegen hat man den im Meere stattfindenden Prozessen mehr abstrakte wissenschaftliche, als allgemein nützliche Bedeutung beigemessen. Dieser weitverbreitete Standpunkt wurde durch die Schlussfolgerungen aus den Funden von Schwefelwasserstoff im Schwarzen Meere und Hypothese Brandts über die Denitrifikation erschüttert.

Die Rolle der Bakterien, als eines bedeutenden Faktors, wurde mehr und mehr klar, es stellte sich auch deutlich heraus, dass die Tätigkeit der Bakterien in engem Zusammenhange mit der Entwickelung des tierischen und pflanzlichen Lebewesens in den Wasserbassins steht. Um einen kurzen Überblick über die Untersuchungen, die der Mikrobiologie des Meeres gewidmet wurden, aufzustellen, muss man vor allem die Arbeiten von Certes an Bord des "Travailleur" und "Talisman" im Jahre 1883 und solche von B. Fischer an Bord des "Moltke" 1886 über die Bakterien des Atlantischen Ozeans erwähnen. Diese haben zum ersten mal gezeigt, dass sogar die Tiefen von 3000–5000 m. Bakterien enthalten, was bis dahin kaum für möglich gehalten wurde. Es wurde auch trotz der Unvollkommenheit der Technik eine gewisse Vorstellung über den Reichtum des Meeres an Bakterien konstatiert. Diesen Arbeiten folgten solche der Schwarzmeer-Expedition von Andrussow, die, wie ich schon erwähnte, das Vorkommen von Schwefelwasserstoff im Schwarzen Meere und seine bakterio-

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logische Natur nachwiesen. Eine Reihe von Untersuchungen seitens der russischen Gelehrten, die auch diese Entdeckung hervorgerufen hatten, erweiterten unsere Kenntnisse über die Entstehung des Schwefelwasserstoffes und die über die Herkunft des schwarzen heil Schlammes. Diesen Untersuchungen schliessen sich auch solche von Russel (1892–93) im Golf von Neapel und im Atlantischen Ozean bei Woods Hole, Mass. Nur flüchtig berührt die Bakterien des Polarozeans Levin auf der "Antarktik" (1889). Der vorzeitige Tod des Dr. Buchmanns, Mitgliedes der "Valdivia-Expedition" unter Leitung Chun's (1899) hat uns sicherlich von viel Interessantem aus dem Bakterienleben der Meerestiefen beraubt.

Die Arbeiten, welche auf Anregung von Brandt unternommen wurden, erstreckten sich auf die Gewässer des Baltischen Meeres, solche von Minervini an Bord des "Fürst Bismarck" auf den Atlantischen Ozean. Minervini hat, meines Erachtens, nur das bewiesen, dass an Bord eines Passagierdampfers die bakteriologischen Beobachtungen kaum möglich sind. In den Jahren 1901-1903 wurden bakteriologische Untersuchungen während der "Gauss"-Expedition unter Leitung von Drygalski von Gazert geführt, wobei ammoniakbildende Bakterien gefunden wurden, nicht aber solche, die Nitrifikationsprozesse hervorrufen. In den Jahren 1906–07 untersuchte Gräf während der "Planet"-Expedition die Verbreitung der denitrifizierenden Bakterien im Indischen Ozean.

Das wäre alles, was über die Expeditionen, welche sich mit Meeresbakterien beschäftigten, gesagt werden konnte. Darüber habe ich ausführlich in meiner 1914 in Petersburg veröffentlichten Arbeit "Die Untersuchungen über die Bakterien des nördlichen Polarozeans" (russ.) berichtet. Diese Untersuchungen, welche 1906 an Bord des "Andrei Perwoswanny" der unter Leitung Breitfuss stehenden Murman-Expedition ausgeführt wurden, erbrachten den Beweis über die Organismen, die im Polarmeere im Kreislauf des Stickstoffs und des Schwefels tätig sind; sie konstatierten auch die Anwesenheit von denitrifizierenden Bakterien, die bei der ziemlich niedrigen Temperatur das Nitrat zerlegen. Diese Beobachtungen bestätigen nicht die Hypothese von Brandt, welche, wie bekannt, den Planktonreichtum in den nördlichen Meeren durch das Fehlen in diesen Meeren von aktiven denitrifizierenden Bakterien erklärte. Es wurden von uns Bakterien nachgewiesen, welche den gasartigen Stickstoff an der Oberfläche der Algen fixieren, und somit, wie es uns scheint, der Beweis dafür erbracht, dass der Reichtum der nordischen Meeren an Planktonorganismen, einerseits, durch die Tätigkeit der stickstoffixierenden Bakterien, andererseits—und das ist die Hauptsache—durch das grössere Löslichkeitsvermögen der Kohlensäure im kalten Wasser des Polarozeans, als in den dem Equator nahe liegenden warmen Gewässern, erklärt werden muss. In dieser Arbeit berücksichtigte ich auch solche Bakterien, die Sulphate reduzieren und dadurch auf einigen Stellen des Polarozeans an der Bildung von "blue mud" teilnehmen. Ich behandle auch in meinem Werke die Purpurbakterien, welche in dem 15 m. tiefen Reliktensee Mogilnoje schon 2 m. vom Boden aufwärts eine Mikrozone (Niveau) bilden, und den Schwefelwasserstoff sich nicht höher verbreiten lassen.

Man muss somit anerkennen, dass die ersten mehr oder weniger ausführlichen, wenn auch nicht vollkommenen, Untersuchungen über die Meeresbakterien von der Murman-Expedition an Bord des "Andrei Perwoswanny" gemacht worden sind und dass durch die Beobachtungen die Einheitlichkeit der Prozesse sowohl im Erdboden, wie auch in den Wasserbecken, wo diese Prozesse vorwiegend auch im Grundboden vor sich gehen, festgestellt ist. In allen Fällen vollstrecken sich diese Prozesse durch die Tätigkeit der Organismen, die sich angepasst haben, oder durch die *sui generis* marinen Formen, die ohne Nachteil die Konzentration des Salzgehaltes von 3 Prozent im Wasser vertragen.

Einige von meinen Beobachtungen wurden später bestätigt, andere aber, wie z.B. die über das Vorkommen von nitrifizierenden Bakterien, mit Zweifel empfangen (Lipmann 1922). Dies alles beweist nur, dass es noch unendlich viel Unbekanntes in den Meerestiefen sich findet und es rechtfertigt die Worte des Ozeanographen Thoulet: "Aujourd'hui l'océan cache encore bien des mystères."

Anlässlich der verschiedenen Fischereifragen wurde im Jahre 1922 unter Leitung Knipowitsch eine Expedition zur Erforschung der Tiefen des Asowschen und des Schwarzen Meere ins Leben gerufen. Ich nahm an diesen Untersuchungen als Bakteriologe teil und habe mir als Aufgabe gestellt, die Aufklärung der Frage über die Bildung von Schwefelwasserstoff im Schwarzen Meere und über die Prozesse im Kreislauf des Schwefels. Die Arbeiten wurden in den Sommermonaten an Bord des "Besstraschny" und des "Suchum" ausgeführt, das gesammelte Material in meinem Laboratorium in Leningrad unter Teilnahme meiner Assistenten Frau A. Egoroff und Frl. M. Ontschukoff bearbeitet. Wertvolles Material erhielt ich auch von W. Nikitin, dem Leiter der biologischen Anstalt zu Sebastopol.

Schon die Andrusow's Expedition hat festgestellt, dass die Tiefen des Schwarzen Meeres, welche bis zu 2300 m. reichen, durch den Schwefelwasserstoff vergiftet sind und dass nur eine verhältnismässig dünne Schicht von etwa 200 m. frei von diesem für das Leben schädlichen Gas ist. Dadurch ist die starke untere Schicht des Schwarzen Meeres von jeglichem Leben frei; ausgeschlossen müssen, gewiss, nur die Bakterien sein.

Mich interessierten hauptsächlich, erstens, der Bodengrund, wo sich das eigentliche Bakterienleben konzentriert, und, zweitens, jene Wasserschicht, die an der Grenze der Schwefelwasserstoffausbreitung und also auch der Lebensgrenze lag.

Das Wasser des trichterförmigen Beckens des Schwarzen Meeres ist von 200 m. abwärts mit Schwefelwasserstoff durchdrungen, dessen Konzentration, je mehr man sich dem Boden nähert, wächst und dicht am Boden in einer Tiefe von 2300 m. 6.54 ccm. auf ein Liter Wasser, wie es die Untersuchungen Lebedintzew's zeigten, erreicht. Die Beobachtungen unserer Expedition zeigten, dass in der Nähe der Küste der Schwefelwasserstoff aufwärts bis zu einer Tiefe von 175 m. reicht, dagegen im offenen Meere seine Grenze näher der Oberfläche in etwa 125 m. Tiefe liegt. Somit muss die Lebensgrenze höher im offenen Meere

und niedriger an den Küsten liegen und die Oberfläche der mit Schwefelwasserstoff gefüllten Schicht muss einer konkaven Linse ähnlich sein.

Über die Bedingungen der Schwefelwasserstoffsbildung besitzen wir die Arbeiten von Zelinski und Brussilowski, die einen spezifischen Mikroorganismus beschrieben haben, nämlich *Bakterium hydrosulfureum ponticum*, welcher den Schwefelwasserstoff sowohl aus den Sulphaten, als auch aus der bei aeroben und anaeroben Prozessen entstehenden organischen Substanz produziert. Die Natur dieses Organismus bleibt bis heute noch sehr wenig aufgeklärt. Indem ich mir die Frage, durch welchen Organismus der Schwefelwasserstoff gebildet wird, gestellt hatte, habe ich in erster Linie, gestützt auf Wahrscheinlichkeit des Vorhandenseins in dem Becken des Schwarzen Meeres von anaeroben Bedingungen, meine Aufmerksamkeit auf das Suchen eines anaeroben sulphatreduzierenden Organismus gelenkt. Ich hoffte einen Organismus zu finden, welcher ähnlich der *Mikrospira* von Beyerinck und van Delden, die ich im Polarozean entdeckt hatte, sein könnte.

In der Tat, ist es mir auch gelungen, in einem anaeroben Medium, das nur Schwefel in der Form von Sulphaten enthielt, die Bildung von Schwefelwasserstoff zu erreichen, wobei einige Formen von Bakterien sich entwickelt haben. Aus einer Reihe von Versuchen konnte ich feststellen, dass die Schwefelwasserstoffbildung durch eine Vibrione hervorgerufen wird, welche ähnlich der erwähnten *Mikrospira* ist. Diese Vibrione konnte während der Entwickelung im anaeroben Medium, wo die Quelle des Stickstoffs, Asparagin, und Schwefel (in der Form von Sulphaten) vorhanden war, ein sehr beträchliches Quantum, etwa 0.3–0.5 gm. pro 1 Liter Schwefelwasserstoff, bilden.

Neben diesem energischen Schwefelwasserstofferzeuger wurden im Schlamme des Schwarzen Meeres noch mehrere andere Bakterienformen, welche Schwefelwasserstoff bei Anwesenheit von Eiweisstoff erzeugen, entdeckt; diese erwiesen sich aber bei weitem weniger energisch in Bezug auf die Schewefelwasserstoffbildung, als die schon erwähnte *Mikrospira*. Die meisten von ihnen sind kaum fähig das *Bleipapier* zu schwärzen.

Das lässt mich annehmen, dass die Entstehung von Schwefelwasserstoff im Schwarzen Meere sich hauptsächlich auf der Reduktion der Sulphate basiert. Diese Reduktion findet eine weite Verbreitung im Meere sowohl auf dem Gebiete der biologischen, als auch der mit diesen eng zusammenhängenden hydrologischen und geologischen Erscheinungen.

Über den Bildungsvorgang des Schwefelwasserstoffes im Schwarzen Meere haben wir ein vollkommen klares Bild vor uns; dagegen, was das Asowsche Meer anbelangt, so erscheint die Frage über die Bildung von H_2S von einem sporadischen Charakter. Dabei übt diese Erscheinung einen sehr grossen Einfluss auf die biologischen Vorgänge in diesem sehr seichten Bassin, dessen maximale Tiefen nur zwischen 15-16 m. schwanken aus. Gewöhnlich beobachtet man im Asowschen Meere keinen Schwefelwasserstoff in den Schichten unmittelbar über dem Boden, abgesehen davon, dass im Grunde des Bodens dieser Gas bestimmt vorhanden ist, weshalb auch der Boden in vielen Stellen unter dem Namen "stinkiger Schlamm" bekannt ist. Es treffen sich aber Perioden, wo das Wasser

dieses Meeres, besonders in seinem westlichen Teile, einen stark ausgeprägten Schwefelwasserstoffgeruch aufweist, die oberen Wasserschichten sind sogar vollkommen des Sauerstoffes entbehrt. In solchen Perioden sterben die Fische und schwimmen tot auf der Oberfläche. Diese Erscheinung steht, meinem Ermessen nach, in engem Zusammenhang mit der starken Produktion von Schwefelwasserstoff.

In der Tat, haben meine Beobachtungen das Vorkommen der *Mikrospira* nachgewiesen, welche bei der Reduktion der Sulphate den Bodenschichten einen schwarzen Anstrich verleiht. Die Tätigkeit dieses Organismus verläuft im anaeroben Medium und lebendige Mikrozone, die sich über dem Boden streckt, trägt dazu bei, dass es sich im Grundboden schon in geringerer Tiefe günstige anaerobische Bedingungen entwickeln. Bei der geringen Tiefe des Meeres dringt der Sauerstoff bis zum Boden in das Meer hinein, wozu in grossem Masse die Wellen, welche die Wasserschichten von der Oberfläche bis zum Boden durchwühlen, beitragen. Es genügen aber einige Tage stillen Wetters: das Durchwühlen hört auf und es tritt die Bildung von Schwefelwasserstoff ein, wobei die Entwickelung der Schwefelwasserstoffbakterien derart steigt, dass das Wasser nicht mehr für Lebenszwecke brauchbar wird. Hier tritt mit aller Deutlichkeit die Abhängigkeit der Reduktion der Sulphate von den anaeroben Bedingungen auf, da auch hier die Hauptrolle der *Mikrospira* zukommt.

Wie ich schon erwähnt habe, verbreitet sich der entstehende Schwefelwasserstoff von der Tiefe aufwärts und erreicht verschiedene Höhen. Theoretisch betrachtend, nach Egunoff, muss man annehmen, dass an der Grenze, bis zu welcher dieser Gas im Wasser verbreitet ist, eine Mikrozone (Platte) gebildet wird. Egunoff's Annahme basiert sich auf Experimenten mit dem Schlamm im engen Zylinder, wobei in einiger Entfernung vom Boden sich eine Mikrozone bildete. Es war von Wichtigkeit aufzuklären, ob ähnliche Bakterienansammlungen in den natürlichen Gewässern vorkommen und ob sie imstande sind der weiteren Verbreitung des Schwefelwasserstoffes Schranken zu stellen. Die erste Beobachtung darüber wurde von mir im Jahre 1906 bei der Untersuchung der Bakterien des nördlichen Polarmeeres im Reliktensee Mogilnoje auf der Insel Kildin an der Murmanküste gemacht. Man konstatierte hier in 1 Liter Wasser *folgende* Quanten von Schwefelwasserstoff:

in	13 m. Tiefe	0.133 ccm.
"	14 " "	6.181 "
"	15 " "	17.673 "
"	15¾ " "	22.913 "

An der oberen Grenze dieser Schwefelwasserstoffschicht befindet sich eine Schicht von hellrotem Wasser, welche grosse Ansammlungen von Purpurbakterien: *Chromatium vinosum* und *C. minutissimum* aufweist. Über dieser Rosaschicht trifft man keinen Schwefelwasserstoff mehr. Daraus folgt die wahrscheinliche Bedeutung dieser Bakterienschicht für die Oxydationsprozesse.

Falls dieselbe Erscheinung auch im Schwarzen Meere an der Grenze der Verbreitung des Schwefelwasserstoffes stattfindet, so müsste man erwarten,

auch hier entweder farblose Oxydationsorganismen, wie es Egunoff annimmt, welche bei Laboratoriumversuchen in engen Zylindern sich unter dem Bodenschlamm bilden, oder Ansammlungen von Purpurbakterien anzutreffen. Indem ich mich auf die Erfahrung aus dem Mogilnoje-See und auf die theoretischen Erwägungen stützte, hoffte ich jene oder andere Oxydationsorganismen an der Grenze der Schwefelwasserstoffschicht zu finden. Zu diesem Zwecke wurde zuerst an der Beobachtungsstelle die obere Grenze des Schwefelwasserstoffs festgestellt, wobei zur Kontrolle auch die planktonologischen Funde herangezogen wurden; dann wurden Wasserproben über je einen Meter entnommen und sowohl auf- wie abwärts dieser Grenze. Es sammelten sich 20–30 Wasserproben aus einer Vertikalserie.

Derjenige, der im Meere gearbeitet hat, kennt, was eine solche Serie von 140–250 m. an Arbeit und Zeit erfordert. Die Proben wurden mikroskopisch behandelt, es wurden Farbpräparate angefertigt, Aussaaten gemacht sowohl auf verschiedene Nährboden, wie es bei Thiobakterien üblich ist, als auch auf für die marine Bakterien gebräuchlichen organischen Medien.

Die negativen Resultate sind trotz ihrer Unbestimmtheit doch nicht ohne Bedeutung, da sie die anderen Forscher zu weiteren Untersuchungen und zwar mit Methoden, die besser sind, als die meinige, anregen können.

Im Mogilnoje-See, wo eine Mikrozone vorhanden ist, habe ich dieses auch positiv bewiesen, im Schwarzen Meere dagegen, konnte ich, trotz meiner grössten Mühe, keine Mikrozonen weder von Thiobakterien, noch Schwefelbakterie, oder deren Ansammlungen nachweisen. Im Gegenteil, in den Aussaaten von Schlammproben entwickelten sich diese Organismen, dabei aber wies nichts darauf hin, was die Annahme von der Existenz einer Mikrozone berechtigen könnte. Die Mikrozone könnte im Schwarzen Meere sehr dünn sein und ist möglicherweise bei Entnahme von Wasserproben über je einen Meter unberührt geblieben. Wie es auch sei, es bleibt dem künftigen Forscher diese Frage zu bejahen, oder zu verneinen. Im letzten Falle würde die Theorie neu bekräftigt und die Tatsache aus dem Mogilnoje-See würde nicht gesondert da stehen.

Es giebt noch Erwägungen hydrologischen Charakters, die die Frage der Oxydation des Schwefelwasserstoffs im Schwarzen Meere durch Bakterien in ein ganz anderes Licht stellen. In der Tat: in einem hohen Glaszilinder mit Wasserschichten von verschiedener Konzentration entsteht keine vertikale Zirkulation. Es fehlt solche Zirkulation auch im Mogilnoje-See, welcher Schichten verschiedener Konzentration aufweist. In solchen Fällen bildet sich als Regel an der Grenze der Schwefelwasserstoffschicht eine Mikrozone aus Bakterienansammlungen, ein, so zu sagen, bakteriales Zone-Niveau. Im Schwarzen-Meere sind die physikalischen Verhältnisse weit andere. Es ist ein tiefes trichterförmiges Becken mit beständigem Zufluss von süssem Wasser aus den Flüssen, wodurch die oberen Schichten stark bräckisch werden und dadurch die Bedingungen für eine Zirkulation geboten. Die Forschungen unserer Expedition haben die Bedeutung für die Existenz im Küstengebiete einer vertikalen Zirkulation bis zu einer Tiefe von 150–200 m. erbracht, d.h. bis zur Grenze, wo die Schwefelwasserstoffzone ihren Anfang nimmt. Falls diese Annahme richtig ist, so müssten

die Schwefelwasserstoffoxydationsprozesse im Schwarzen Meere nicht auf einer biologischen, sondern auf einer chemischen Basis beruhen.

Demnach haben die Schwefelwasserstoff oxydierenden Bakterien im Schwarzen und Asowschen Meeren eine weite Verbreitung, wobei ihr Hauptsitz vorwiegend im Bodenschlamm ist. Im Asowschen Meere z.B., an den Stellen, wo man stinkigen Schwefelwasserstoff enthaltenden Schlamm antrifft, findet man Ansammlungen von Purpurbakterien (*Lamprocystis roseo persicina*) und besonders die farblose *Beggiatoa*. Daneben lassen sich ohne besondere Schwierigkeiten aus solchen Bodenproben Thiobakterien ausscheiden. In solchem Schlamme, in einen schmalen Glaszilinder, unter eine Wasserschicht übertragen, entwickelt sich eine typische Egunoff's Mikrozone (Platte). Somit ist laut unseren Beobachtungen die Verbreitung von Schwefelwasserstoff-und Thiobakterien im Asowschen und Schwarzen Meeren unbestreitbar konstatiert, obwohl wir diese stets nur in Kulturen bei Bodenaussaaten erhalten haben. Diese Organismen aus dem Wasser auszuscheiden oder zu beobachten ist uns niemals gelungen. Obgleich es nicht ausgeschlossen ist, dass im Asowschen Meere auf seichten Stellen und in Fällen, wo hier im Wasser die Schwefelwasserstoffbildungsprozesse vorgehen, solche Organismen auch an die Oberfläche gebracht werden können.

Ferner muss ich auf die weite Verbreitung in dem Grundboden des Asowschen Meeres von denitrifizierenden Bakterien hinweisen, welche den Schwefel als Energiequelle ausnützen. Somit sind für den Kreislauf des Schwefels in beiden von uns untersuchten Meeren mehrere Erreger zur Stelle: einerseits die Sulphate reduzierenden und Schwefelwasserstoff bildenden Organismen, andererseits solche, die diesen Schwefelwasserstoff oxydieren und Schwefel und Schwefelsalze bilden, und, endlich, solche, die den Schwefel als Energiequelle zur Reduktion der stickstoffhaltiger Salze verwenden. Jede Gruppe von Organismen, die an diesen Prozessen teilnimmt, zählt mehrere morphologisch differenzierten Formen. Eine spezielle Charakteristik dieser gedenke ich in einem anderen Vortrag zu geben, hier will ich nur in grossen Zügen von dem Verlauf der bakteriologischen Prozesse in diesen Meeren Kenntnis geben. In Zusammenhang mit der Schwefelwasserstoffbildung kann auch die Frage über die Fermentation der Zellulose, welche in grossen Mengen in den Meerestiefen abgelagert ist, behandelt werden. So, z.B., trifft man an der Kaukasus-Küste am Boden nicht nur die Reste vom Phytoplankton, sondern auch solche von Landpflanzen, wie Buchenlaub, etc. Bei Zersetzung scheidet die Zellulose, wie bekannt, Wasserstoff aus. Da dieser Wasserstoff, wie Hoppe-Seyler gezeigt hat, die Reduktion der Sulphate bewirkt, so muss ein gewisser Zusammenhang zwischen der Reduktion der Sulphate und Zersetzung der Zellulose in den Meeresbecken vorhanden sein.

Unsere Untersuchungen sind aus diesem Grunde dahin gerichtet worden, dass man in erster Linie nach den Bakterien, durch welche die anaerobe Zellulosezersetzungsprozesse am Boden bedingt werden, zu suchen. Zu diesem Zwecke wurden Bodenproben von einigen Gramm genommen und auf Nährboden Omeljanski ausgesät. Es zeigte sich, dass bei allen Versuchen, bei denen die Grundproben nahe den Küsten oder aus Tiefen nicht über 300 m. stammten, man die Zersetzung der Zellulose mit Bildung von Gasen feststellen konnte;

dagegen ergaben die Proben aus grösseren Tiefen und auch aus den Tiefen über 2000 m. stets negative Resultate. Diese Versuche lassen mit Sichercheit schliessen, dass (an den Kaukasischen und Krimschen Küsten des Schwarzen Meeres) die zellulosezersetzenden Organismen ausschliesslich nur auf Küstengebiet anwesend sind, da mit steigender Tiefe die Schwefelwasserstoffzone beginnt, wo keine Schwefelwasserstofferreger anzutreffen sind.

Diese Beobachtungen können als Beweisgrund für die Annahme gelten, dass, nämlich, wie Beyerinck meint, die Schwefelwasserstoffbildung zu den direkten Prozessen der Reduktion von Sulphaten gehört.

Darum, muss der Zellulosezersetzungsprozess im Schwarzen Meere als ein sehr wichtiger Faktor in der Oekonomie dieses Wasserbeckens angesehen werden. Als Erreger dieses Prozesses kommt ein sporentragender Bacillus in Betracht, mit dessen Studium ich gegenwärtig beschäftigt bin.

Im Asowschen Meere findet die Zersetzung der Zellulose ebenfalls auf allen Stellen statt und deren Erreger sind sowohl aerobe, wie anaerobe Formen.

Nun tritt eine andere Frage vor dem Forscher auf—nämlich die Frage über den Kreislauf des Stickstoffes. In Wirklichkeit, bei dem enormen Reichtum der Meere an Pflanzenleben ist dort ein grosser Mangel an Stickstoffverbindungen vorhanden. Ich möchte hier auf die Arbeiten von Brandt hinweisen, der ein interessantes Bild über die Verteilung der stickstoffhaltigen Verbindungen in einigen Meeren gegeben hat. Eine weite Verbreitung der denitrifizierenden Bakterien in den Meeren ist bereits durch die Arbeiten von Baur, Gran, Feitel, durch die meinige und die meiner Schüler bewiesen; diese Frage erweckt also keinen Zweifel mehr. Etwas anders steht es mit der Frage der Nitrifikation, worüber noch keine bestimmte Meinung gefasst ist. Einige, wie Tomsen, berichteten über Funde von nitrifizierenden Bakterien in den Meeren; andere wieder, wie Nathansohn und Gran, bestritten dieses für die von ihnen untersuchten Meeresbezirke (Nathansohn für den Golf von Neapel, Gran für die Norwegischen Küstengewässer). Ich persönlich hatte Gelegenheit in den Grundproben des nördlichen Polarozeans die Bakterien von Winogradski zu konstatieren, leider aber will mein verehrter Kollege Lipmann nicht mal die Möglichkeit des Vorkommens dieser Organismen in den Meeren zugeben. Wenn ich richtig verstehe, so hat Lipman selbst die Gelegenheit gehabt eine Nitrifikation bei Bodenaussaaten aus dem Stillen Ozean auf Winogradski's Nährboden zu beobachten. Darin ersehe ich keinen Widerspruch mit meinen Funden von nitrifizierenden Bakterien aus dem nördlichen Polarmeer. Es bleibt nun noch fraglich, ob diese Organismen im Meereswasser vorkommen. Ich gebe zu, dass auch diese Frage im positiven Sinne beantwortet werden wird, falls man zu Aussaaten das Grundwasser nimmt, d.h. Wasser, welches freischwimmende Grundpartikel enthalten kann. Dagegen, was die ganze Wassermasse anbetrifft, so bezweifle ich es ebenso, wie es mein verehrter Kollege Lipmann tut. Um diese Frage zu beantworten, unternahm ich mehrere Aussaaten von Wasser- und Grundproben aus dem Asowschen und Schwarzen Meeren. Dabei konstatierte ich stets, dass in Wasserproben die Nitrifikationsbakterien fehlten, dagegen in den Grundproben im Asowschen Meere überall vorhanden waren; im Schwarzen Meere aber traf ich

dieselben nur in der Küstenzone, d.h. in der Zone, wo keine Verseuchung durch den Schwefelwasserstoff war. Dies ist einleuchtend und benötigt keine weitere Erklärung.

Ich komme, also, zu der Überzeugung, dass in dem Bodenschlamm, d.h. da, wo sich Ammoniak bilden kann, nitriterzeugende Prozesse vorgehen können, und dieses muss bei der Aufklärung der Meeresphysiologie berücksichtigt werden.

Ich will hier noch die sehr interessante Hypothese von Reincke über die Symbiose zwischen den Algen und den Stickstoffixierenden Organismen—*Azotobacter* und *Clostridium*—erwähnen. Meine Untersuchungen im Polarozean haben ein sehr weites Vorkommen dieser Bakterien an der Oberfläche der *Laminaria* und *Fucus*, sowie anderer Organismen konstatiert. Auch im Asowschen Meere konnte ich diese Organismen an der Oberfläche der Algen feststellen.

Wir können also gegenwärtig, wie es mir scheint, mit grosser Überzeugung feststellen, dass das Meerwasser keine Hindernisse der Entwickelung von solchen Bakterien stellt, welche ähnlich denen sind, die wir auf jedem Schritt auf dem Trockenen treffen und deren Bedeutung in Bezug auf das Gedeihen des Pflanzlichen Lebens nicht unbedeutend ist und uns die Möglichkeit giebt, über die dazu vorhandenen Quanten von Stickstoff zu urteilen, die den marinen Formen ebenso notwendig sind, wie auch den Landformen. Durch die Tätigkeit der Mikroorganismen wird der nötige Bedarf der Algen an Stickstoff gedeckt, obwohl die Stickstoffverbindungen im Meerwasser nur in minimalen kaum wahrnehmbaren Quanten nachzuweisen sind.

Das Studium von bakteriologischen Prozessen in den Meerestiefen führt uns, also, zum Schluss, dass der Kreislauf der Elemente im Meere stets in engem Zusammenhang mit der Bakterientätigkeit steht und dass den Meeresbakterien nicht weniger Aufmerksamkeit gewidmet werden muss, als den Bodenbakterien.

UEBER THIOBAKTERIEN DER SALZSEEN[1]

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Bekanntlich erfolgt der Oxydationsprozess des Schwefelwasserstoffes in den schwefeligen Verbindungen einerseits mittels Schwefelbakterien, deren Physiologie von S. N. Winogradski erforscht ist, andererseits aber mittels sogenannter Thiobakterien, die zuerst von Nathanson und Beyerinck und dann von Jakobsen beschrieben worden sind (letztgenannter gab noch ergänzende Details über dieselben)—und zuletzt lieferte der interessante Fund des *Thiobacillus thioxydans* Lipmann's Wachsmann's Joffe ein klar ausgesprochenes Bild dieses Oxydationsprozesses.

Alle diese Arbeiten, so wichtig dieselben ihrem Wesen nach auch sind, können doch nur als Vorarbeiten gelten, die nur den Beginn der Klärung von physiologischen Eigenheiten der Gruppe Bakterien darstellen, welche den Schwefel oxydiren und eine ausserzellige Ablagerung desselben bilgen, und deshalb steht auch in der Sache der Thiobakterien-Erforschung noch sehr viel Arbeit bevor.

Der vorliegende Bericht über die Untersuchungen, welche noch bei Weitem ihr Ende nicht gefunden, hat den Zweck doch einiges Licht über Bakterien zu bringen, die den Oxydationsprozess in den schwefeligen Verbindungen in den Salzseen der Krim hervorrufen. Die erwähnten Arbeiten begannen unabhängig von den Forschungen Trautweins, welche dieselbe Frage berühren, und liefern im allgemeinen nur eine Bestätigung seiner Angaben über die Physiologie dieser Bakteriengruppe,—doch muss man im Auge behalten, dass meine Forschungen anderen Formen dieser Gruppe gewidmet waren.

In der Krim sind einige Salzseen mit Soole von recht hoher Konzentration, die zum Sommerschluss eine Höhe von 26–30° Bohme erreicht; der Boden dieser Seen ist mit einem vortrefflichen schwarzen Schlamm (heilwirkend) bedeckt, der als Resultat einer ganzen Reihe komplizierter biologischer Prozesse erscheint. Zugleich mit einer Menge purpurner und farbloser schwefliger Bakterien kommen hier auch Thiobakterien vor, welche in ihren einigen spezifischen Eigenheiten dem *Thiobacillus thioparus* ähnlich sind. Beim Nachforschen gerade nach dem soeben genannten Organismus wurden die vorerwähnten Bakterien im Schlamm aller von mir erforschten Seen (es waren über 10) gefunden.

Ihre Anwesenheit in den oberen Schichten des Schlammes (Moor) zu konstatieren, war nicht schwer. Hierfür war ausreichend, eine Aussaat auf das Nährmedium vorzunehmen, welches Jakobsen für den *Thiobacillus thioparus* für geeignet hält. Nach zwei bis drei solcher Aussaaten konnte man sicher sein, dass in

[1] Presented before the International Congress of Plant Sciences, Section of Bacteriology, Ithaca, New York, Aug. 20, 1926.

diesem Medium, vermischt mit anderen Bakterien, sich ein Organismus in Rohkultur befindet, der aus Schwefel die Sulphate bildet. Das Absondern desselben in Reinkulturen bietet noch einige Hindernisse, da die feineren Details dieser Methode noch nicht ausreichend durchgearbeitet worden sind, doch gelingt auch solches relativ ohne besondere Schwierigkeiten.

Die charakteristische Eigenschaft aller dem *Thiobacillus thioparus* ähnlichen Organismen besteht darin, dass an den Kulturen am Agar, welchem der Schwefel in der Gestalt von schwefligsaurem Natrium ($Na_2S_2O_3$) beigefügt wird, um die kleinen Kolonien des ausscheidenden Organismus eine Ablagerung von pulverähnlichen Schwefel sich bemerkbar macht, und diese bietet vollkommen die Möglichkeit, diese Gruppe Bakterien von den anderen zu unterscheiden.

In meinen heutigen Mitteilungen beschränke ich mich lediglich auf die Formen, welche aus den Saki und Mainaki Seen unweit Eupatoria und Tschokrak See unweit Kertsch ausgeschieden wurden.

Sämmtliche ausgeschiedenen Formen sind Aerobien, die sich durch schnelle Beweglichkeit auszeichnen; es sind winzig kleine Stäbchen, nicht Sporentragende; sie unterscheiden sich eines vom anderen durch die Beständigkeit der Zellengrössen, so dass sie als Formen gelten können, wenngleich morphologisch dem *Thiobacillus thioparus* auch nahestehend, doch mit demselben nicht identifizieren. Ich nenne: *Bakterium* (*Thiobakterium*) *Beijerinckii* die aus dem Saki See, *Bakterium* (*Thiobakterium*) *Nathansonii* die aus dem Mainaki See, und *Thiobakterium Beijerinckii* f. *Jakobsenii* die aus dem Tschokrak See entnommenen Formen.

Thiobacillus thioparus ist von Beijerinck aus dem Meerwasser (an der Küste Hollands von Beijerinck und an der Küste des nördlichen Eismeeres von Issatschenko u.s.w.) entnommen. Formen, welche in Wasserbassins mit hoher Konzentration leben, sind bisher noch nicht beschrieben worden. Deshalb erschien es höchst interessant, ein klares Bild darüber zu schaffen, inwieweit die Wirkung der Konzentration des Mediums, welche auf die verschiedenen Quantitäten des Chlornatrium zurückzuführen ist, auf die Entwickelung dieser Organismen sich geltend macht, und soll hierbei das Quantum des vom ihnen erzeugten Sulphates als Masstab dienen.

TABLE 1. SULPHAT-QUANTITÄTEN NACH 1 MONAT BEI DIVERSEN MENGEN NaCl IN PER CENT
(in mgr. auf 100 ccm. des Nährbodens Jakobsens)

	0	2%	4%	8%	16%	20%	24%	32%
Saki See	..	1160	1366	1071	471	· 32	23	..
Mainaki See	..	534,8	507,4	460,1	8,5	..	..	..
Tschokrak See	..	786	502	666,6	256,5	123,3	89	..

Auf diese Art zeigten die Organismen der Saki und Tschokrak Seen ein grösseres Anpassungsvermögen betreffs der bedeutenden Mengen Chlornatrium im Vergleich zu den Organismen des Mainaki Sees. Gleichzeitig liess sich bei ihnen allen bei Erhöhung der Konzentration eine Verminderung des Oxydationsprozesses wahrnehmen, wobei bei 32% NaCl ein absolutes Fehlen von Sulphatbildungen sowohl bei den Formen des Saki Sees, als auch bei denjenigen aus

dem Tschokrak See konstatiert werden konnte. Ausserdem findet keine Entwickelung aller ausgeschiedenen Formen im Medium bei niedrigem osmotischen Druck statt; die grösste Menge von Sulphatbildungen liess sich bei 2–4% NaCl beobachten. Dieses alles lässt mich vermuten, dass die gefundenen Formen von Meeresformen stammen, welche im Meerwasser bei Konzentration 2–3%NaCl ihr Dasein führen. Da die genannten Seen aus dem Meere entstanden, indem sie nur durch unbedeutende sandige Scheidewände vom Schwarzen und Asowschen Meer getrennt sind, was verhältnissmässig unlängst (blos einige Jahrhunderte) vor sich gegangen sein muss, so ist eine solche Annahme durchaus begründet. In dem See, wo die Konzentration, wie ich hervorhob, gewöhnlich eine sehr hohe ist, wesentlich höher als 10–12 Prozent, ist es natürlich, dass die Oxydationstätigkeit dieser Organismen sich in einem deprimirten Zustande befindet, doch—wenn auch langsam— vor sich geht.

Da bei den soeben gezeigten Versuchen das Optimum der Entwickelung bei 2–4 Prozent NaCl festgestellt wurde, so wurden die folgenden Experimente, welche der Erforschung einiger physiologischen Eigenschaften dieser Organismen dienten, im Nährboden Jakobsens bei einer Konzentration von NaCl 2 Prozent durchgeführt.

Im Falle einer reinen Kultur solcher Organismen, die sich in streng anorganischem Medium entwickeln können und welche die Energie der Oxydation des Schwefels ausnutzen, ist es von Interesse, die Frage zu lösen, wie das Verhalten dieser Gruppe von Thiobakterien insbesondere zum Stickstoff organischer oder anorganischer Herkunft sei.

Die Resultate solcher Versuche sind aus dem Folgenden ersichtlich:

TABLE 2. SULPHAT-MENGEN AUF 100 ccm. IN MGR. NACH 30 TAGEN
(Nährmedium Jakobsen's mit 2% NaCl)

NH_4Cl	KNO_3	$(NH_4)_3PO_4$	Pepton	Asparagin
197,9	238,8	205,6	450,4	303,6

Diese Versuche haben gezeigt, dass die betreffenden Organismen als prototrophe ihr Dasein führen können und dass zu gleicher Zeit ihr Übergang zur metatrophen Ernährung, ohne ihre Entwickelung zu verlangsamen, ihre Oxydationseigenschaft zu verstärken geeignet ist.

Dabei ist hervorzuheben, dass bei einer Vergrösserung (gewiss bis zu den bestimmten Grenzen) der Menge der organischen Substanz, eine Erhöhung der Energie ihrer Tätigkeit, welche durch das Quantum der Sulphatbildungen bemessen wird, erzielt werden kann.

TABLE 3. SULPHAT-MENGEN BEI VERSCHIEDENEN QUANTITÄTEN ASPARAGIN AUF 100 CCM.

0,02 mgr.		0,04 mgr.	
1. Versuch	2. Versuch	1. Versuch	2. Versuch
651,5	721,4	702,0	809,2

Aus meinem kurzen Bericht stelle ich fest, dass: die Anzahl der Formen von Thiobakterien bedeutend grösser ist, als es bisher angenommen war; morphologisch sie sich ohne Zweifel genügend scharf unterscheiden die Formen, welche sich in den Salzseen der Krim entwickeln und welche, wie die Beobachtungen zeigen, sich in einem Medium mit verhältnissmässig hoher Konzentration entwickeln können, wahrscheinlich Marineformen sind. Indem sie als metamorphe Organismen erscheinen, können sie auch paratrophe Lebensweise führen, d.h. als fakultativ paratroph erscheinen.

Aus den obigen Angaben geht hervor, dass die Beobachtungen Trautweins über die günstige Wirkung der organischen Substanz auf die Oxydationsfähigkeit der ausgeschiedenen Bakterien sich bestätigen. Soweit es sich schliessen lässt, sind die Formen, an denen die hier geschilderten Versuche gemacht wurden, doch nicht immer dieselben und entwickeln sich gewöhnlich unter besonders eigenartigen Bedingungen, d.h. sind es andere, als die von Trautwein beschriebenen. Daraus die Folgerung, dass die fakultative Paratrophie eine Eigenschaft vieler Bakterien ist.

Womit ich mich aber mit Trautwein nicht einverstanden erklären kann, das ist mit seiner Behauptung, dass eine Absonderung des Schwefels in dem Umkreise der Kolonien in reinen Kulturen nicht stattfindet. Meine Beobachtungen an den Reinkulturen der Krimschen Seeformen geben allen Grund zur Behauptung, dass die Schwefelabsonderung auch in den Reinkulturen geschieht und nach der Formel

$$Na_2S_2O_3+O=Na_2SO_4+S$$

vor sich geht, wobei der Säuregehalt (Hydrogen Jonkonzentration) des Mediums wächst.

[Mehr ausführliche Daten, siehe: Bulletin de l'Institut Hydrologique, No. 21, 1928, Leningrad.

OXIDATION-REDUCTION IN RELATION TO BIOCHEMISTRY[1]

W. MANSFIELD CLARK

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When carbon is converted to CO or when CO is converted to CO_2, it is natural to say that an *oxidation* has occurred. When this process is reversed and there occurs a reduction in the degree of oxidation, it is natural to say that a process of *reduction* has taken place.

Now it so happened that the discovery of oxygen was associated with experiments of such fundamental importance to chemistry in general and to physiological chemistry in particular that the formation and reduction of oxides became a type for a species of reaction having many varieties. For example, we now recognize two states of iron, called ferrous and ferric, the corresponding ions differing only by one electron as expressed by the relation:

$$\underset{\text{(Ferric)}}{Fe^{+++}} + \text{electron} \rightleftarrows \underset{\text{(Ferrous)}}{Fe^{++}}$$

Although no oxygen appears in this expression, we call the transformation Ferrous→ferric an oxidation, and the reverse a reduction, because it is possible to maintain a schematic orientation of the relations by referring to the type as follows:

$$\begin{array}{lll} 2FeO + O_2 & \longrightarrow & Fe_2O_3 \\ \quad\text{ferrous oxid} & & \text{ferric oxid} \\ \Big\downarrow \text{solution in HCl gives} & & \Big\downarrow \text{solution in HCl gives} \\ 2Fe^{++} & & 2Fe^{+++} \\ \text{ferrous ion} & & \text{ferric ion} \end{array}$$

Or consider the indigo vat. As the cloth leaves the vat the accompanying indigo white turns blue because of its exposure to oxygen. Surely this is an oxidation. But the organic chemist has shown that the isolated indigo white differs from the isolated indigo blue only in having two more hydrogen atoms per molecule

$$\text{Indigo blue} + H_2 \rightarrow \text{Indigo white}$$

The simplest assumption is that the oxygen attacks the hydrogen of indigo white. Therefore when we speak of the dehydrogenation of indigo white we schematically refer to the type reaction and call the dehydrogenation an oxidation and the hydrogenation a reduction.

All this is, of course, familiar. However, it is not always remembered that

[1] Presented before the International Congress of Plant Sciences, joint session of the Sections of Bacteriology and Physiology, Ithaca, New York, Aug. 20, 1926.

there is a sharp distinction between the postulation of a mechanism for convenience in classification and the representation of a mechanism as an actuality. The craving to comprehend the realities has been the incentive to those ceaseless searches for mechanism, accounts of which have crowded the literature for a century.

To praise the progress made in this direction does not offset the fact that an air of uncertainty surrounds its more philosophic aspect nor does it offset the astonishing fact that an abundance of experimentation directed by this search for mechanism has left untouched some of the most elementary problems.

We therefore turn to an entirely different mode of approach in which the postulates used in classification are recognized as pure conveniences and in which there is no immediate concern for mechanisms.

There are certain conditions defined by thermodynamics under which a system of reacting substances liberates the maximum of energy it is capable of setting free. When devices can be found for measuring this free energy, its quantitative evaluation furnishes a solid basis for the discussion of many important problems. Consider, for instance, the reduction of ferric ions to ferrous ions by hydrogen at a definite acidity. The classic device for measuring the free energy change is represented by Figure 1.

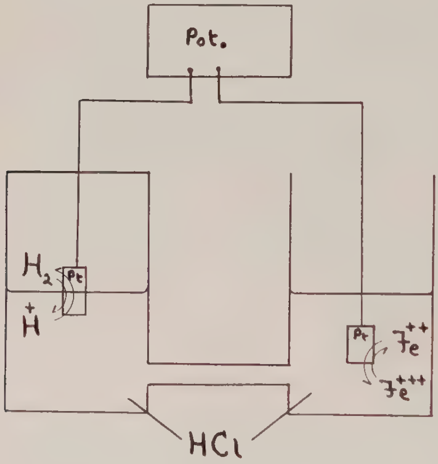

FIG. 1.

A solution of HCl is divided into two half-cells. In one half-cell is placed an equimolecular mixture of ferrous and ferric chlorides, which we shall assume furnish equal parts of ferrous and ferric ions. In the other half-cell are the hydrogen ions from the hydrochloric acid and molecular hydrogen at one atmosphere partial pressure. In each half-cell is a platinum electrode. The two half-cells are connected by a tube made comparatively narrow to approximately isolate two processes. When the electrodes are put into metallic connection the cell delivers current which is accompanied by the oxidation of hydrogen to hydrions in the one half-cell and the reduction of ferric to ferrous ions in the other half-cell. Now we know from Faraday's law that a definite *quantity* of electricity accompanies the transformation of one gram mol of ferrous ions to one gram mol of ferric ions and that in general this same *quantity* of electricity accompanies all such transformations involving what is known as one chemical equivalent. This is the *quantity factor* in the energy involved. Our interest next centers on the intensity factor of this energy of reduction. In this instance it is measured in volts—not while the cell is running down, for that would involve some complexities—but by exactly balancing the electromotive force of the cell against an oppositely imposed, external electromotive force.

To be specific, let us say that there is in the one half-cell an equimolecular mixture of ferrous and ferric ions, in the other there is hydrogen at one atmosphere pressure, and in both there is a normal concentration of hydrogen ions.

The electromotive force of the cell, as measured by the potential difference between the electrodes is 0.75 volts. This is a quantitative expression for the driving force with which hydrogen at one atmosphere pressure, restrained by a normal hydrogen ion concentration, tends to convert an equimolecular mixture of ferrous and ferric ions toward the entirely ferrous state.

Now suppose we replace the ferrous-ferric system by an equimolecular mixture of indigo carmine and leuco indigo carmine, keeping the other half-cell under the conditions specified above which are those of the so-called normal hydrogen electrode. We measure the electromotive force or potential difference and find it to be 0.29 volts. This is a quantitative measure of the driving force with which hydrogen at one atmosphere restrained by a normal hydrogen ion concentration tends to convert an equimolecular mixture of indigo carmine and leuco indigo carmine toward the entirely reduced state at pH 0.

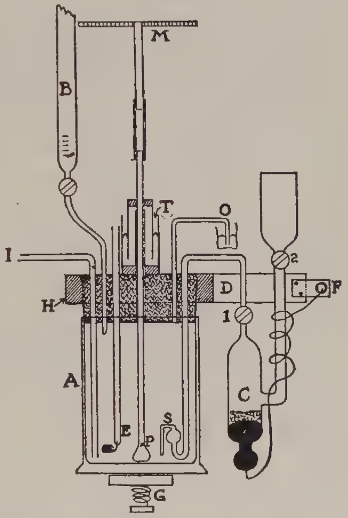

Fig. 2.

Having referred these specific iron and indigo half-cells to the common standard (the normal hydrogen half-cell) we know that the difference $0.75-0.29=0.46$ volts is a measure of the driving force with which an equimolecular mixture of indigo and indigo white at normal hydrion concentration (pH 0) tends to convert an equimolecular mixture of ferrous and ferric ions to the ferrous state. Conversely 0.46 volts is a measure of the driving force with which this particular iron system tends to oxidize this indigo system.

It is at once perceived that for all cases wherein this device is applicable the relative oxidation-reduction intensities of systems can be measured and for convenience expressed in potentials (volts).

These methods of expression and of measurement have been extensively applied in inorganic chemistry, but it is only within the last 6 or 7 years that they have been systematically applied to organic systems.

One of several reasons for the backwardness of the organic applications is the following. Most organic systems have groups which dissociate as acids or bases. Now suppose the anion of indigo white exerts a different reducing tendency from the undissociated material. Obviously the utmost confusion will result unless the hydrion concentration of the solution is put under control so as to establish a definite degree of dissociation in any given case.

Thus, systematic studies of oxidation-reduction in organic systems have had to await the development of a familiarity with acid-base equilibria.

Since I am trying to develop the theme without recourse to equations, I shall not describe the scheme which is used to maintain consistency in the tortuous route which has to be followed in formulating the relations found in specific cases. I merely emphasize the importance of hydrion concentration and shall now pass at once to the description of experimental data with the aid of graphs corresponding to the equations.

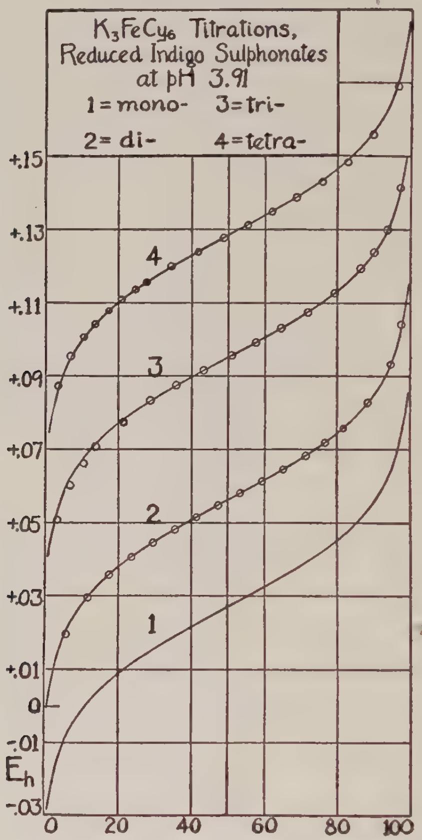

FIG. 3.—Relation of electrode potential, E_h (ordinate) to percentage oxidation (abscissa).

Let a buffer solution be placed in vessel A of Figure 2. After sweeping out the oxygen by means of a stream of pure nitrogen add to the buffer solution a known amount of reduced indigo carmine, keeping its amount so low in relation to the buffer salts that there will be no serious change in pH. Now oxidize the reduced indigo carmine in steps by successively adding small portions of a ferricyanide solution. At each step measure, by means of a potentiometer, the difference of potential between the electrode E and the mercury of the calomel half-cell C.

To avoid technicality let us suppose that the calomel half-cell C is replaced by a normal hydrogen half-cell. It is to the basis of this comparison that our numerical data are brought, and when the "potential" of a system is mentioned the potential of this cell is implied.

If now we chart the potentials, E_h, against the percentages reduction we have the dots of Curve 2, Figure 3. They conform to a type curve.

The data for the trisulphonate and tetrasulphonate also conform to the type-curve. This position of this type-curve in the case of the monosulphonate, is calculated from equally good data taken under other conditions.

The type-curve is asymptotic to the potential axis at 0 per cent oxidation (100% reduction) and 100 per cent oxidation (0% reduction). In other words, there is no definite potential for a pure oxidant or a pure reductant. For a given increment of oxidation or reduction there is least change of potential at 50 per cent oxidation. In other words the system is then most stable and we take the potential at this mid-point as a characteristic. Since the mid-point for the tetra system is positive to that of the trisulphonate system, the tetra system is oxidizing with respect to the tri. In the actual interaction of two such

systems we must, of course, take account of the total quantities of the materials in each. Suppose there were such a predominance of the 50–50 mixture of the tetrasulphonate and its reductant that a small amount of added leuco indigo carmine could not make a large percentage change in the tetra system. The tetra system would stay near +.13 volts and the disulphonate would be oxidized to a simultaneous potential near +.13 volts. This means practically complete oxidation of the disulphonate (Indigo carmine).

If we plot such curves for a large number of organic systems we are in a position to deal quantitatively with the direction and extent of various oxidation-reduction reactions.

In Figure 4 are grouped a number of indophenols.

Note the monotony of form and the differences in level of the mid-points.

Unfortunately the relative positions of the curves may not remain the same when the pH is changed. For instance Figure 5 shows the change of potential of the half reduced system in each of three cases, when the pH of the solution changes. The inflexions of the curves are in each instance due to the passing of a zone of pH below which some one group has its ionization suppressed and above which this group suffers complete ionization.

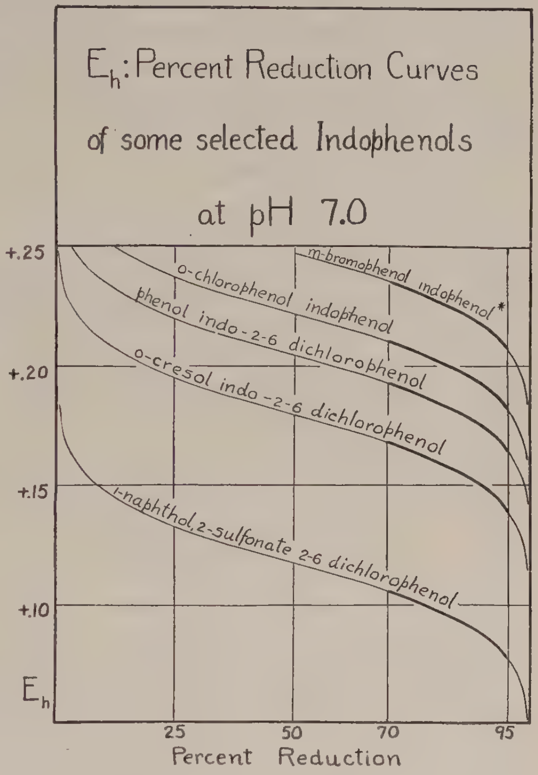

FIG. 4.

In dealing with organic systems we have then to consider the three coordinates: percentage oxidation, pH, and potential. Up to this point we have considered separately first the case where pH is kept constant and the potential varies with percentage oxidation, and second the case where percentage oxidation is kept constant and the potential varies with pH. Synthesizing these, we have in the case of 2,6- dibromo indophenol the isometric drawing of Figure 6. In this are shown again the S-curves obtained when pH is constant and the potential varies with percentage oxidation. When the percentage oxidation is 50 the potentials vary with pH, as shown by the central curve extending from the upper right hand corner to the lower left hand corner. At any other constant percentage oxidation the curve would be parallel to this central curve.

Since the curves for constant pH values all have the same form and since

their mid-points are defined by the central curve of the second type the latter curve is sufficient for practical uses. Knowing from this second curve the potential for 50 per cent oxidation at any given pH, we estimate from the 1st type curve, or by Table 1, the potential at any other percentage oxidation and at the given pH value of the solution.

In Figure 7 are curves for several different systems at 50 per cent oxidation. It is evident that the variation of potential with variation of pH follows no uniform rule. This is because no two systems possess the same acidic or basic dissociation constants. Consequently, as pH shifts in value each compound, in a different manner, has its own trend. There is, therefore, nothing to do but to develop a description of each system experimentally and to abandon hope of any common description.

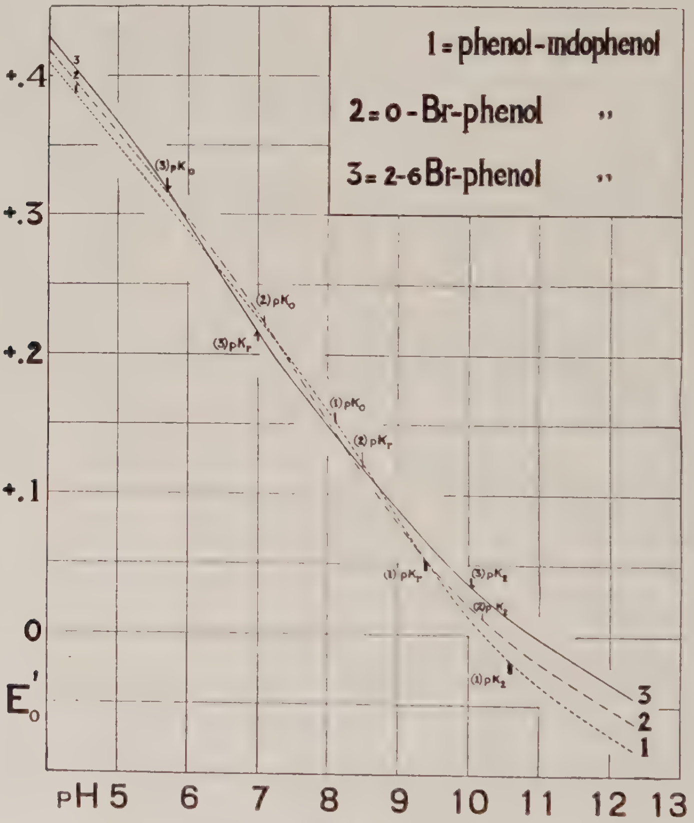

FIG. 5.

Note particularly in Figure 7 that the half-reduced ferro-ferri cyanide system is oxidative with relation to the half reduced indophenol system at pH values greater than pH 4 and reductive at pH values less than 4. Note also the remarkably high slope of the methylene blue system in acid solutions and its wide

sweep to a comparatively low slope in the pH zone of greatest physiological importance.

Without the aid of equations it is very difficult to describe the conduct of those systems the orienting curves of which are shown in Figure 8. Benzidine, for instance, on oxidation forms a pale yellow oxidation product. It is the combination of this with the colorless reductant that gives the initial highly colored product which the organic chemist calls a meriquinone and the biochemist uses in color tests. In the formation of a colored meriquinone only certain species of oxidant and reductant combine. The proportions of these species which are present are determined by pH and consequently the pH value of the solution is of fundamental importance to the color test. But within the zone of pH where the meriquinone can form, the formation at stages of oxidation less or greater than 50 per cent, may withdraw so much of the residual excess oxidant or reductant as to cause a large distortion in the curve relating potential to percentage oxidation. In other words Table 1 is not applicable. The curves of Figure 8 alone are therefore not adequate as similar curves are in the cases previously described. The distortion mentioned does, however, provide a means of determining the extent of meriquinone formation in specific cases and helps us experimentally to complete the descriptions of such systems.

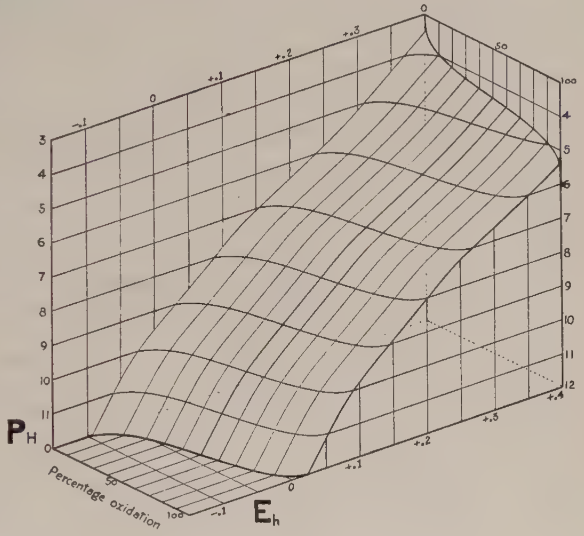

FIG. 6.

Having accomplished this quantitative description, we can say that these systems are extremely complex. Furthermore, their instabilities have serious consequences not perceived in previous studies. This combination of complexity and instability renders unsafe the practical use of benzidine, paraphenylene diamine and similar reagents.

Through the work of Biilmann and his students in Copenhagen, LaMer and Baker at Columbia, Conant and his students at Harvard, and my associates, Cohen, Gibbs, and Sullivan, there are now available descriptions of a considerable number of systems.

We, of the Hygienic Laboratory, have been interested in developing a series of oxidation-reduction indicators which can be used in biochemistry in a manner analogous to the use of acid-base indicators. Figure 9 shows the curves for 50 per cent oxidation of several of the indicators we have studied. Knowing from such a chart that the potential of half reduced methylene blue at pH 7.4 is

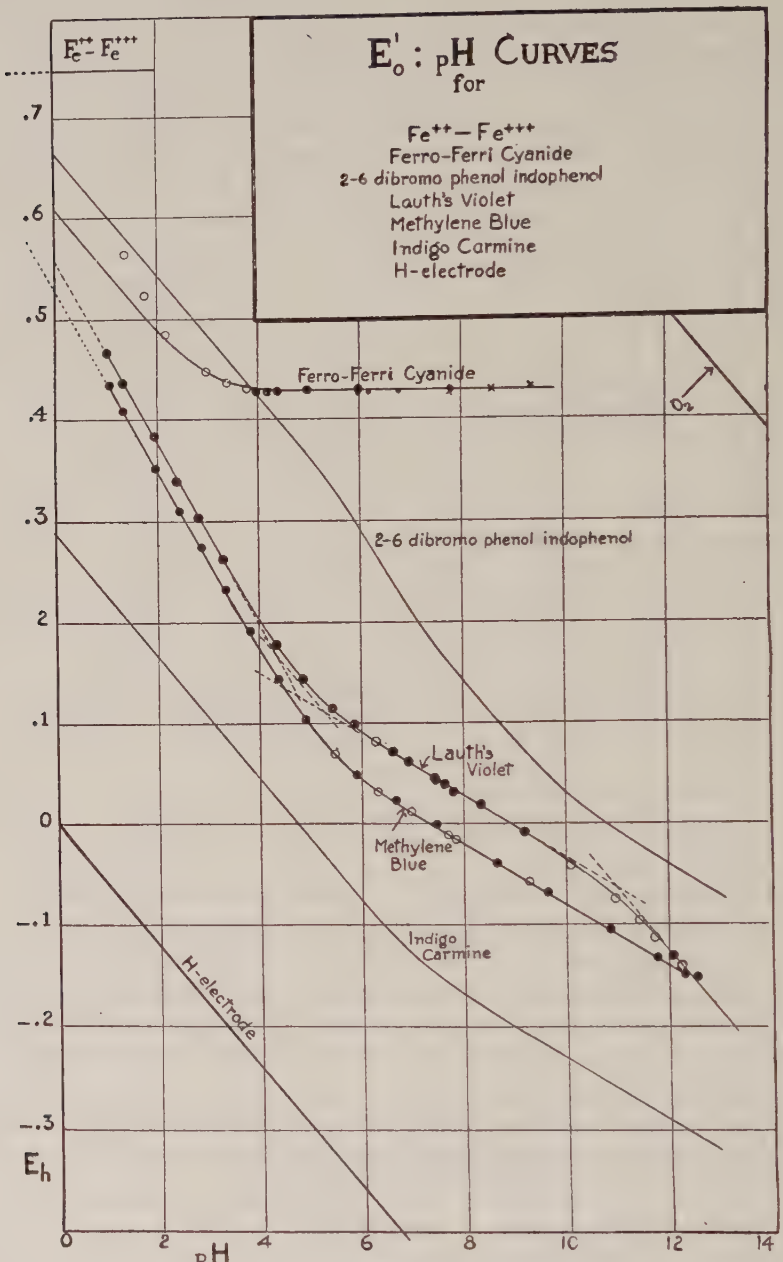
E'o : pH CURVES for
Fe++ — Fe+++
Ferro-Ferri Cyanide
2-6 dibromo phenol indophenol
Lauth's Violet
Methylene Blue
Indigo Carmine
H-electrode
Fe++ - Fe+++
Ferro-Ferri Cyanide
2-6 dibromo phenol indophenol
Lauth's Violet
Methylene Blue
Indigo Carmine
H-electrode
O2
Eh
pH

FIG. 7.

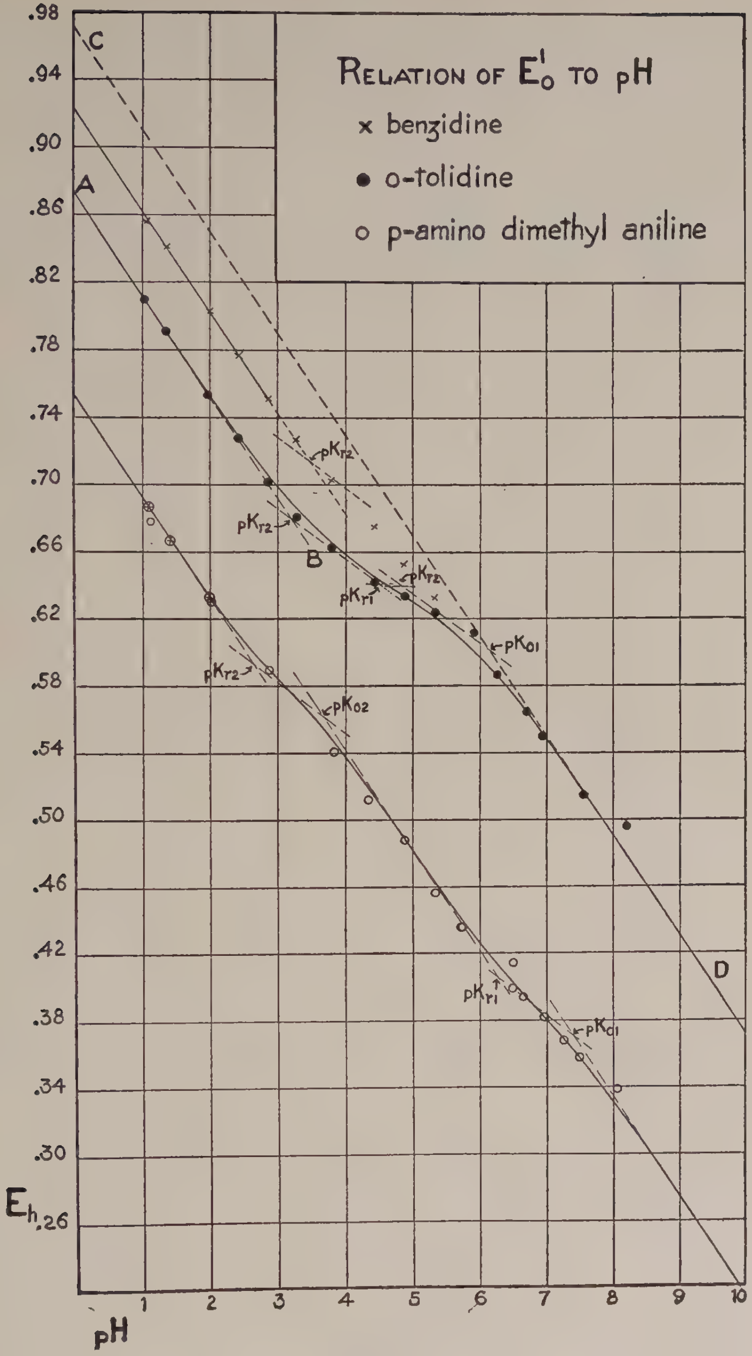
Relation of E_0' to pH
× benzidine
• o-tolidine
o p-amino dimethyl aniline
A
B
C
D
pK_{r2}
pK_{r1}
pK_{o1}
pK_{o2}
$E_{h,26}$
pH
.98
.94
.90
.86
.82
.78
.74
.70
.66
.62
.58
.54
.50
.46
.42
.38
.34
.30
.26
1
2
3
4
5
6
7
8
9
10

FIG. 8.

0.002 volt we can calculate from Table 1 that at the same pH the state of 95 per cent reduction of methylene blue corresponds to a reduction potential of −0.040 volt. We also know that, if a solution maintains this potential, not only will methylene blue be at the state of 95 per cent reduction but all the indophenols (Curves 7–9) will be completely reduced and indigo carmine (Curve 2) will be practically completely in the oxidized form.

TABLE 1. TO CALCULATE THE POTENTIAL OF AN OXIDATION-REDUCTION INDICATOR AT A GIVEN PERCENTAGE OXIDATION (OR REDUCTION) AND GIVEN pH WHEN THE POTENTIAL OF THE SYSTEM AT 50% OXIDATION (OR REDUCTION) IS KNOWN FOR THE GIVEN pH ADD THE PROPER VALUE GIVEN BELOW. (CORRECTIONS ARE EXACT FOR 30°C.)

Oxidation %	Reduction %	Correction volts
0.1	99.9	−.0902
1	99	−.0600
5	95	−.0384
10	90	−.0287
20	80	−.0181
30	70	−.0111
40	60	−.0053
50	50	.0000
60	40	+.0053
70	30	+.0111
80	20	+.0181
90	10	+.0287
95	5	+.0384
99	1	+.0600
99.9	0.1	+.0902

Such relations concern equilibrium states. The subject as developed to this point has no more to do with rates of reaction than it has to do with mechanism, or the moon.

However, it is important to certain applications to note that these indicators, in contradistinction to certain other reagents, adjust their equilibrium states instantly when acted upon by any system capable itself of establishing a definite electrode potential. Futhermore, there appear in the data themselves, in contradistinction to any implication which may be placed in the concepts used in reaching these experimental data, evidence that reduction of these indicators consists in the transport of electrons in pairs. When hydrogen is found in the isolated reductant in excess over the hydrogen of the oxidant, it is because the process of isolation has driven hydrogen ions into the compound or because the initial acid-base equilibria was such as to produce this effect.

Now as we ordinarily view the oxidation-reduction reactions accomplished by living cells, we recognize few cases comparable with those discussed. Some of the plant pigments now being studied abroad, we may expect to hear, are somewhat comparable. There are undoubtedly in some plants quinol-quinone systems very directly comparable with those for which there are now exact data. Neill is describing as "reversible" the oxidation-reduction of the hemotoxins of pneumo-cocci, of *B. Welchii* and of tetanus bacilli.

But since quantitative measurement of equilibrium states is the keynote of our theme, it is not appropriate to speculate but only to hope that there will be found in nature a sufficient number of cases to make the direct and simple application of the new methods eminently useful. But we should not be too sanguine of encountering an abundance of simple and directly mastered cases. The more important types of change seem to belong to that category which Conant calls irreversible and which superficially, at least, appear to be clear cases of hydrogen or oxygen transport, without the involvement of detectable electron transport.

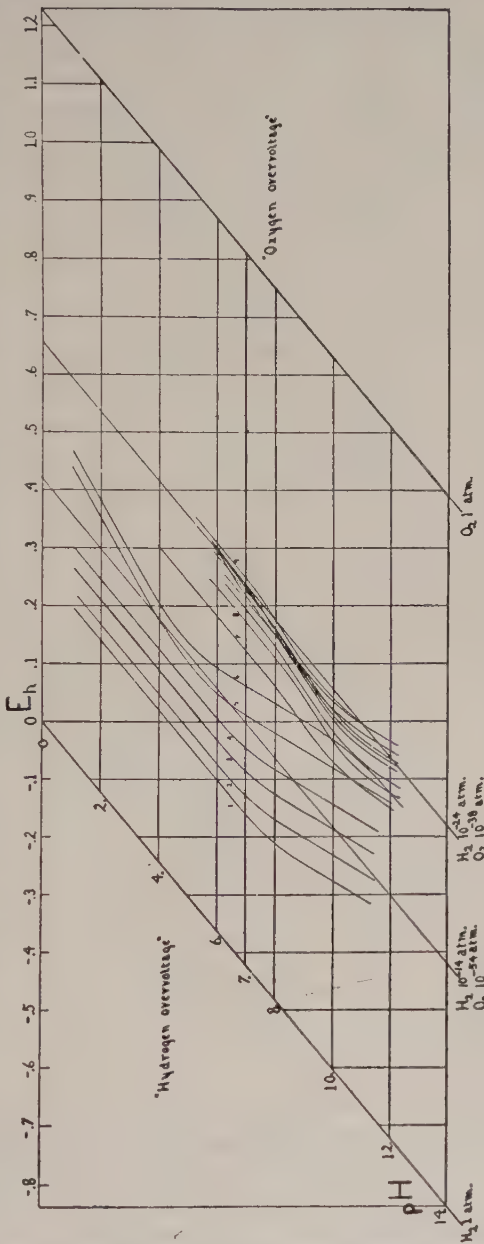

Fig. 9.—E'_0:pH curves of a few oxidation-reduction indicators—1. Indigo monosulphonate; 2. Indigo disulphonate; 3. Indigo trisulphonate; 4. Indigo tetrasulphonate; 5. Methylene blue; 6. Lauth's violet; 7. 1-naphthol 2-sulphonic acid indophenol; 8–9. Indophenols.

It is therefore remarkable that the favorite reagents of biochemist, cytologist, and bacteriologist have been the nicely reversible and electromotively active indicators which at first appear to have little in common with the phenomena they are supposed to detect.

Is there then any evidence suggesting that the systems in the living cell establish direct connection with the transport of electrons in indicator systems by involving such transports themselves? Please bear in mind that we are not compelled to put the question in this form but that it may be helpful to do so.

In Figure 10 are shown the changes of electrode potential with time of washed yeast cells at different pH values. No indicator and no system known to give definite electrode potentials had been added. Now that we know the range of potentials within which several indicators are reduced we would expect these

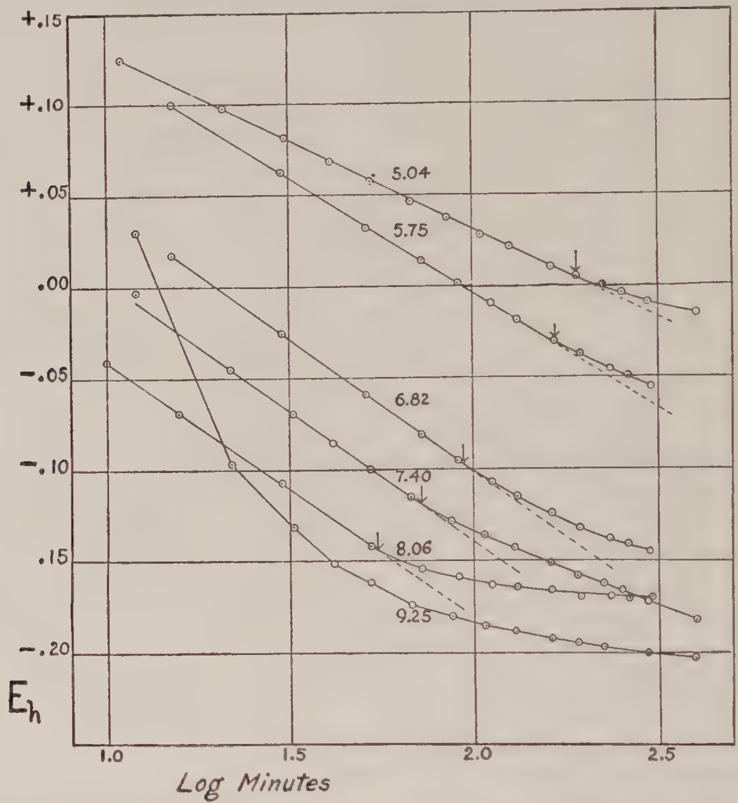

FIG. 10.—Time: potential curves of yeast cell suspensions buffered at the indicated values of pH, and under anaerobic conditions.

indicators to be reduced by the cell suspension in a sequence of the same order as that of their positions on the potentiometric scale. This I found to be the case in 1919, after Gillespie had shown that bacterial cultures produce definite drifts of electrode potential. With our additional data for new indicators Voegtlin, Johnson, and Dyer confirmed the correlation in part.

If a sufficiently large amount of an indicator be present the potential will tend to be stabilized or, as we say "poised" for a time in the zone characteristic of the indicator. An example is shown in Figure 11. By means of the curve relating percentage oxidant of methylene blue to potential at the given pH we

can calculate the percentage oxidation at any point on Curve A. In this case it turns out that the reduction follows the rate of a monomolecular reaction.

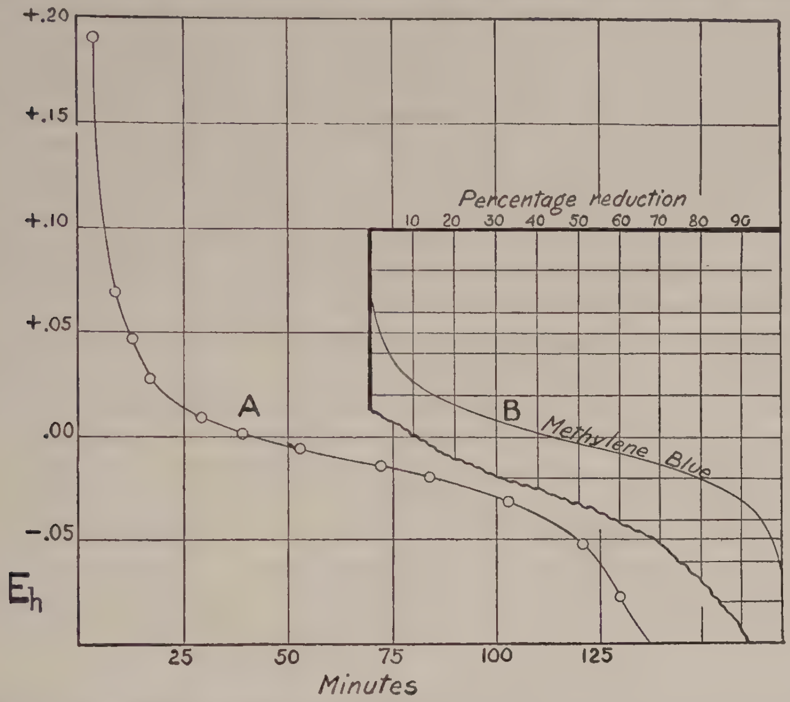

FIG. 11.—Comparison of methylene blue titration curve, B, with the time: potential curve, A, of a suspension of yeast cells containing methylene blue.

Figures 12 and 13 show the time potential curves revealing details in the effects of metabolites, on the one hand, and Hopkins's glutathione, on the other. For discussion see Cannan, Cohen, and Clark, 1926.

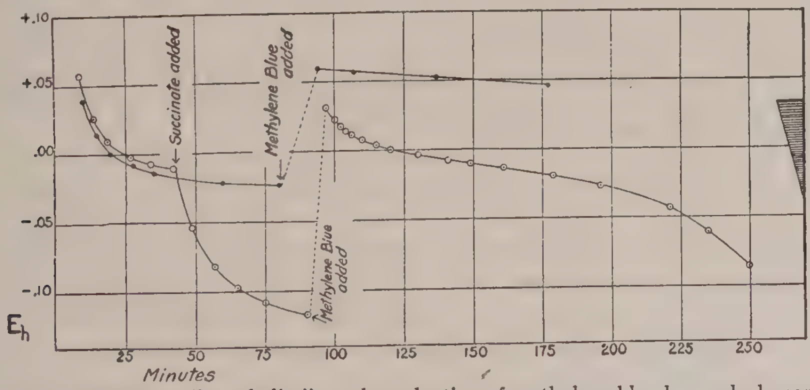

FIG. 12.—Effect of a "metabolite" on the reduction of methylene blue by washed yeast cells.

It is now perceived that we have at hand a new and powerful method of investigating biological oxidation-reduction irrespective of any theoretical interpretation.

Returning now to Figure 9 let me explain the boundaries. We seldom have to deal with solutions more acid than pH=0 or more alkaline than pH=14. These fix the upper and lower boundaries. Next, a solution that is reduced to such an extent that it will support one atmosphere of hydrogen without further decomposition of water in the presence of a catalyst such as platinum black should have the potential of the hydrogen electrode. With change of pH this follows the line at the left. The energy of the decomposition of water to one atmosphere pressure of hydrogen and one atmosphere pressure of oxygen is known. Hence we can calculate the position of the right hand line which indicates the potential at any pH which would occur were the oxidation intensity sufficient to decompose water with the aid of a catalyst and liberate oxygen at one atmosphere.

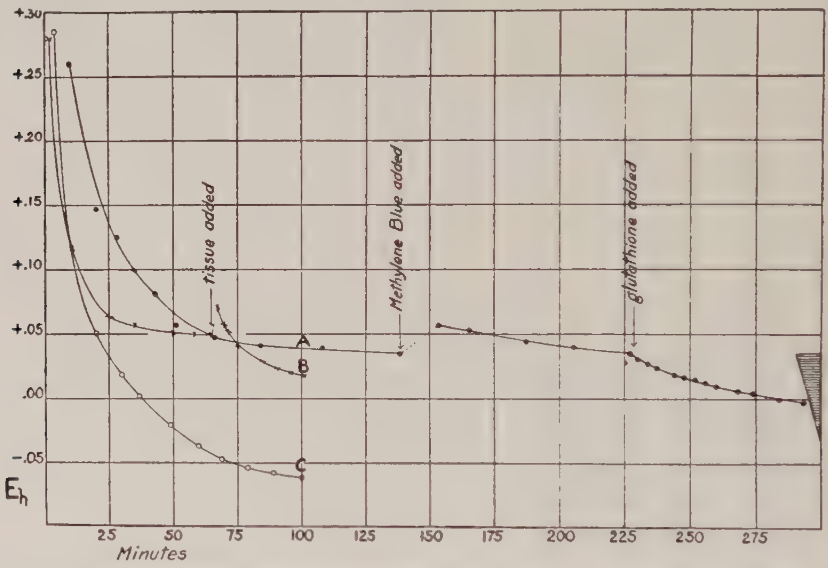

FIG. 13.—Curve A—Effect of glutathione on the time: potential curve of washed, boiled cells in the presence of methylene blue. Curve B—Time: potential curve of glutathione-methylene blue mixture before and after addition of boiled, washed yeast cells. Curve C—Time: potential curve of suspension of yeast cells containing initially, oxidized glutathione.

For the sake of simplicity assume in what follows that pH is maintained a 7.0.

Experimentally it is found that under anaerobic conditions the potentia of a bacterial culture, starting from near the center of the figure, becomes progressively more negative and reaches different levels according to the species. In certain cases the potential passes into the region of hydrogen overvoltage and in such cases hydrogen is evolved. Shall we say that the potentials are due to the evolution of hydrogen or that the hydrogen is evolved because the potential is such that hydrogen should be evolved? There are certain reasons for believing the latter to be the better viewpoint. In the march of the potential toward these highly negative values, it passes the region of the methylene blue

system (Curve 5), that is, metheylne blue is reduced and consequently the region crossed by the line marked $O_2 = 10^{-54}$. This means that when the potential falls on this line the pressure of oxygen in true equilibrium with the system would be 10^{-54} atmosphere. This corresponds to approximately one discrete molecule of oxygen in a cube the edge of which is one million meters.

We would not hesitate to say that an organism growing at this pressure of oxygen is an anaerobe! However, we were careful to state that this represents an equilibrium condition and we know that oxygen needs to be stimulated by very active catalysts before it will enter equilibrium states. Hence the use of methylene blue as an indicator of the absence of oxygen should be conditioned by the proof that the catalysts or the time allowed is sufficient to permit a reasonable approach to an equilibrium state. In short, the decoloration of methylene blue indicates nothing directly regarding anaerobiosis; it indicates the attainment of a definite reduction intensity conveniently expressed in volts. Indirectly it indicates a condition of anaerobiosis when the condition above noted is satisfied. If assurance on this latter aspect can be given there is at hand a definite rejection of the postulate that all bacteria require oxygen. Beijerinck went so far as to say that the requirements of the "microaerophils" are below the power of the chemist to detect. Perhaps so; but the statement is a dangerous one to make in an age when indirect methods measure intangibles.

Since calculations of a similar nature apply to cultures in which indigo is reduced there is established the first step in an argument supporting Pasteur when, on the floor of the French Academy, he answered Gunning's argument against anaerobiosis by stating that he felt convinced of the reality of anaerobiosis because he had observed growth in cultures which had reduced indigo.

The calculation values for oxygen pressures indicated by the lines marked $O_2 = 10^{-54}$ and $O_2 = 10^{-38}$ atmosphere are paralleled by similar calculation values for hydrogen pressures. For purposes of general discussion it is convenient to use the latter values in the form $rH = \log \frac{1}{\text{pressure of } H_2}$, for example, rH14 indicates a reduction intensity corresponding to the calculation value of 10^{-14} atmosphere pressure of hydrogen. Obviously some of these calculation values indicate magnitudes of no actuality and, therefore, it is reasonable to conclude that hydrogen as an entity is not actually concerned in equilibria of systems furnishing these potentials.

A more detailed consideration of this matter has been given in some of our papers. We have reached the conclusion that Wieland's theory of hydrogen transport, while perhaps not disproved in the absolute sense, is one of those which would not have been proposed had the new information been available.

By injecting acid-base indicators, on the one hand, and oxidation-reduction indicators, on the other hand, into a variety of living cells Needham and Needham have found that the conditions in the aerobic cell are those described by a region near the center of Figure 9. In other words the aerobic cell may be regarded as approximately neutral with respect to pH and (for lack of a better

term) approximately neutral with respect to oxidation-reduction intensities. This conclusion had been foreshadowed by our own studies of the reduction of indophenols by cell suspensions and was confirmed by direct electrode measurements of aerated cell suspensions made by Cannan, Cohen, and Clark ('26). It is confirmed by the micro-injection studies of Rapkine and Wurmser. Mrs. Brooks, by observing the reduction or non-reduction of various indicators as they pass through the protoplasm of *Valonia*, comes to the same conclusion. Rapkine and Wurmser used four different cells with large nuclei and conclude that there is no striking difference of potential between the nucleus and the cytoplasm.

At first it appears remarkable that the potential of the aerobic cell should lie so far from the line at the right representing the potentials of aqueous solution in complete chemical equilibrium with water and one atmosphere of oxygen. On second thought it is not strange, because the region between the center and the extreme right is the region in which fall the systems used as oxidative disinfectants, a subject we are now studying by the potentiometric methods.

Thus there begins to appear a semblance of order and correlation in the relations revealed by indicators, on the one hand, and electrodes, on the other. Because of the very precarious nature of biological electrode potentials no safe conclusions from these could be drawn till the indicator systems had been developed and their confirming revelations found.

But how shall we reconcile the potential zone of the aerobic cell with the fact that we have one biological system, at least, which enters into equilibrium with oxygen, namely, the system hemoglobin-oxyhemoglobin? For the present the only answer I know is this: The hemoglobin molecule does not attain complete equilibrium with oxygen and water. If it did it would burn up. The oxygen merely adds in a particular position and is not activated to true combustion vigor. The hemoglobin-oxyhemoglobin-oxygen system is therefore temporary in the absolute sense though we know it to be durable enough for all practical purposes, and in the same sense that the wood of our houses endures for a century when nothing activates the surrounding oxygen.

Under these circumstances the structure of the molecule is not strained to a deep-seated change and may still be subject to that other type of oxidation we have been discussing. Indeed, Conant finds it is, the system hemoglobin-methemoglobin attaining equilibrium states measurable by the potentiometric method and furnishing potentials compatible with those which we have said characterize the aerobic cell.

A consideration of this and other matters indicates new support to the generally accepted view that the participation of oxygen in the more intimate chemistry of the cell cannot be studied by methods having to do with equilibrium states but is a question of rates of reaction influenced by special catalysts under constraint.

To put the matter another way, we may say that the physical chemist would welcome any catalyst which would activate oxygen so as to make the oxygen electrode practical. None has been provided as yet and there is no expectation that it will be found among biological catalysts.

Therefore biological catalysts of oxidation seem to be not at all comparable with the platinum which hastens the attainment of equilibrium involving hydrogen, but they seem to be adapted to the smooth operation of the cell in a zone of potential far below that of the chemist's wet combustion.

In other words, the processes of true oxygenation present 1 type of problem and the processes of true oxidation-reduction in the sense under discussion present a distinctly different type of problem. Appreciation of this removes the basis of certain controversies.

Only last week I saw a manuscript by R. K. Cannan on hermidin. Knowing that the plant physiologists would be interested in this work, I cabled for permission to mention it. Mr. Cannan has graciously granted the permission. As Hass and Hill have recently shown, there occurs in *Mercurialis perennis* a substance called hermidin which oxidizes first to a blue compound called cyanohermidin and then to a yellow compound called crysohermidin.

As Cannan formulates it the steps are Hermidin (colorless)$\rightleftarrows$cyanohermidin (blue), Cyanohermidin$\rightleftarrows$transient oxidant, transient oxidant$\rightarrow$crysohermidin (yellow).

The first two transformations are reversible; the last not. For the first 2 transformations Cannan has succeeded in obtaining a most excellent titration curve, the first case of a 2-stage transformation of 2 equivalents each. In other words, he has at 1 pH level obtained the characteristic equilibrium potential of each system. Furthermore, he has determined the values of this characteristic potential for different values of pH in the case of the first system.

Now it is worthy of note that the compound occurring in the green shoots is hermidin, the reduced compound, even though the photochemical activity of the plant may be evolving oxygen.

I refer you to Cannan's paper for a discussion of this apparent anomaly. For the moment we may regard it as another evidence that the processes of oxygenation are either separated by cell structures or as in the case of hemoglobin, separated in chemical nature from processes of oxidation-reduction of the type which we have been considering.

Without necessarily having committed ourselves to belief in the electron transport theory, we find it useful as a picture to distinguish the type of system which the indicators represent from oxygenations and hydrogenations. Now just as Conant has found the oxygenation of hemoglobin to oxyhemoglobin to be not amenable to electrometric measurement so he has found the hydrogenation of certain ethylene linkages *in vitro* to be not amenable to study by these methods.

Let us grant then that we have clear cases of oxygen transport, clear cases of electron transport, and clear cases of hydrogen transport. Examples referable to each type are known in biochemistry. Examples of their interconnection are known. We have already noted that oxygenations are under constraints which prevent the reactions which we shall call those of electron exchange. Do the constraints of hydrogenations limit the attainment of equilibrium with system of electron exchange?

Thunberg in 1925 published a short note to the following effect. What I presume to be washed cells (origin not known) brought a mixture of succinate and fumarate into equilibrium with methylene blue and methylene white. Since the pH was known to be 6.7, since the percentage reduction of the methylene blue system was measured, and since we had defined potentials for this state of the methylene blue system, Thunberg, by establishing the fact of a simultaneous equilibrium with the succinate-fumarate system, could calculate the potential of this system at the state established by the activity of the yeast cells. Independently, Quastel and Whetham performed the same sort of experiment at pH 7.4 and a higher temperature with bacterial cells but did not make use of our data for methylene blue. This step was taken by Conant who finds essential agreement with Thunberg's potential.

As Thunberg remarks, this is the first time a potential of any significance has been observed with a system involving an ethylene linkage. And as Conant adds, the significance of these results transcend their biological importance and are of fundamental importance to general organic chemistry.

In this recent work there is a strong flavor of the suggestion made by Cannan, Cohen, and Clark that the cell activates by throwing small portions of its metabolites into a condition capable of electron transfer. They were careful to emphasize that this is a mere working hypothesis and that to follow it the immediate task is to isolate definite systems and master the details.

Unfortunately there is displayed in certain of the papers which have recently appeared, a tendency to substitute verbal use of the new terms for the quantitative experimental data they should symbolize. Numerical measure is the essence of our discussion.

There is no content in the use of the terminology until numerical measurements are provided. Now today we find ourselves confronted with a vast literature of qualitative experimentation upon which has been built towering edifices of speculative concepts. I know of no way in which this mass of information can be translated into the terms we employ, except by the processes of isolating particular systems and step by step evaluating their characteristics.

This leads me to remark that the origins of many current concepts can be traced to qualitative observations with color reactions the meanings of which have never been made precise, as, for instance, thermodynamics has given a precise meaning of a kind to the decoloration of methylene blue.

When, therefore, Rapkine and Wurmser say that their observations with cytoplasm and nucleus refute the theory that the nucleus is the seat of oxidation, I am reminded that the origin of this theory lay in the interpretation of certain color reactions which are not yet understood.

Indeed it requires but little circumspection to conclude that the whole subject of the oxidative color reactions, as applied in cytology, has still to be developed. In this task, which almost in its entirety, lies ahead we may well remember the words of Wurmser,—

". . . . In the domain of physiology, thermodynamics has the double privilege of conferring upon problems a high degree of generality and of instructing us regarding the most minute mechanisms of life."

THE MECHANISM OF MITOTIC DIVISION[1]

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Many investigations have appeared since the year 1849, when Hofmeister first published his studies of mitotic division. Especially did interest increase after it was accepted that the chromosomes contain the hereditary factors.

The process takes much the same course in nearly all plant and animal cells so far as its principal features are concerned. Very typical for mitosis are the differentiation, splitting, and separation of chromosomes. All the rest of the phenomena in connection with karyokinesis are subject to many variations which have as yet been shown to have only specific importance.

If we view the process from a teleological point of view, we may say that its function is to divide regularly certain substances between the daughter nuclei, or between the daughter cells, and that above all it is to divide substances which appear to us in the living cell or in fixed preparations as definite morphological constituents of the cell. In addition, those substances are divided which are not morphologically identifiable, and, while this type of division is difficult to recognize, it has clearly a physiological significance. For instance, during mitosis the nucleoli are mostly dissolved and they are formed anew in the daughter nuclei. The possibility is not excluded that their substance may be divided between the daughter nuclei and that it furnishes the material for the formation of new nucleoli. But it is also possible that the substance for the formation of the new nucleoli originates *de novo*, because the material of the original nucleoli is not quite regularly distributed between the daughter nuclei. This can be easily seen in cases in which it is possible to follow the division of this material.

Looking at the mitotic division from rather a teleological point of view, it is also possible to conceive the appearance of the achromatic figure as tending to a division of a specific substance between the daughter cells. This substance, in some respects, is even microchemically different from the remaining cytoplasm. The same might be said concerning the centrioles or the centrosomes. They are hardly to be considered as dynamic centers of division, because in many animals and in most plants the mitotic division takes place in the absence of such organs. In certain cases the centrioles have been regarded as dynamic centers of division, since they divide before the nucleus and take a distinct position at the poles of the achromatic figure. In many cases chloroplasts or pyrenoids behave in a similar way. This is true for the cases in which the cell contains only a single chloroplast or pyrenoid. Such facts indicate that not only chromosomes, but other constituents of the cell as well, may be distributed

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between the daughter cells, that is, between the regions of the poles of the mitotic figure. This distribution happens mostly before the chromosomes begin to move toward the poles. It is especially striking for the chondriosomes in some pollen mother cells, also for the amyloplasts including starch grains. Even crystals or crystalloids move toward the poles, their movement being without any connection with the fibers of the achromatic spindle. Especially clear is the condition in the pollen mother cells of *Larix*. As long as the achromatic figure is not disturbed, no inactive chondriosomes or amyloplasts occur in it. At the beginning they regularly surround the nucleus, afterwards the spindle, and then they accumulate around the 2 or 4 poles. After the first heterotypic division the phragmoplast in *Larix* degenerates and the amyloplasts penetrate into the space occupied formerly by the phragmoplast. When, however, single amyloplasts penetrate earlier into the connecting spindle, they are always oriented parallel with the fibers. The appearance of the fibers, even if they are an artifact in the form in which they are seen in fixed preparations, is based on some real properties of their substance. The accumulation of chondriosomes and amyloplasts around the nucleus and their distribution to the poles of the achromatic spindle cannot be explained by an orientation of the plasma streaming. Their accumulation at the poles is not connected with a corresponding accumulation of cytoplasm in general at the poles, since such an accumulation would in this case require a considerable diminution in the other parts of the cell. After all, a dividing cell does not show generally a common plasma streaming.

That the movement of these particles is a passive one is proved by the circumstance that even accidental inclusions of the cytoplasm are likewise distributed to the poles and translocated in the same way, as, for instance, the chondriosomes. It occurs in the bacterial nodules of some leguminous plants, as in *Lupinus*. In this plant infection, at the outset, takes place with the help of infection threads. Later on the infected cells divide and the symbiotic bacteria are distributed to the daughter cells; thus, later on, all cells are infected without the intervention of infection threads. Before division the bacteria are distributed nearly uniformly throughout the whole cell. They accumulate later around the nucleus; after the appearance of the spindle and before the arrangement of chromosomes in the equatorial plate, the bacteria are pushed to the poles. They disappear completely from the equator of the cell, accumulate at the poles, and remain there until the cell is divided. After this they become scattered irregularly or regularly throughout the daughter cells.

It is possible to explain these and similar movements from a physical point of view in the same manner as Berthold in 1886 tried to explain movements in the cell. When a homogeneous droplet is immersed in a non-homogeneous medium, as, for example, when liquids or dissolving substances diffuse in the medium, then differences in the surface tension of the droplet originate, and it moves in a certain direction. Thus, by a difference in surface tension on 2 opposite sides of suspended particles a movement in a certain direction may be caused.

In the year 1899 I showed by plasmolytic experiments that the physical consistency of the protoplasm changes during mitotic division. During the

prophases a solidification of the protoplasm occurs, and only during the formation of the cell wall does it revert to a more fluid state. That the viscosity of the protoplasm changes during nuclear division may easily be demonstrated by centrifugation experiments. In a centrifuged cell row, resting nuclei will have been pushed against the apocentric cell walls, while spiremes and asters are still situated in the centers of the cells. Nuclei at metakinetic and telophasic stages are only slightly displaced; those nuclei most readily displaced are those in cells in which cell-wall formation is beginning.

The gelification of the protoplasm spreads from the periphery toward the center of the cell and reaches its highest degree in *Hibiscus calycinus*, for example, at the periphery. In some cases this process proceeds so far that a coagulation takes place—probably in connection with a dehydration—forming a ring at the periphery of the cell. Consequently the cell is divided into two systems with a stratified structure so that a movement of suspensions toward the centers of these two systems results. Sometimes it is possible to recognize the gelified protoplasm as a homogeneous substance prominent in the cell.

Besides the movement which occurs in the cytoplasm, there is still another group of movements within the mitotic figure. They take place independently of those which occur in the cytoplasm, although, if the mitotic figure is subjected to centrifuging, both processes may go on undisturbed. Within the figure which has been removed from its normal position the movements of the nucleoli and chromosomes occur in a normal way. In many plants the nucleoli are situated in the equatorial plane, and they either become dumb-bell shaped and divide or else they move without division toward one or the other spindle pole, where they come to rest before reaching the exact pole and then are dissolved. That their solution continues throughout the metaphases is shown by the fact that the nucleoli situated in the equatorial plate are always larger than those which reach the neighborhood of the poles.

If we assume that the nucleoli dissolve from the sides turned toward the poles more intensely than in the equatorial plane, a dumb-bell shaped division must result. If the nucleoli are not placed in the equatorial plate, they assume a club-like or ovoid shape and move toward the poles. When the cells are quickly fixed, for example in a picric-acetic-sulphuric acid solution, the nucleoli moving toward the poles of the achromatic figure are for the most part ovoid or club-shaped. Good material for the observation of this condition is the root tip of *Hibiscus calycinus*. It is possible, therefore, to assume that the movement of the nucleoli toward the poles is caused by a difference in surface tension on the opposite sides of these structures. The nucleoli always arrive in the neighborhood of the poles before the separation of the daughter chromosomes begins.

These facts indicate that the mechanism of the movement of chromosomes toward the poles is quite different from that of the movement of nucleoli. It is very difficult to say in what way the chromosomes arrive at the poles. It is well known that the mechanism of this movement has been explained in different ways, but not one of those explanations can be accepted. A. Fischer expressed the opinion that the chromosomes might be pushed toward the poles by the

growth of the cell and of its included cytoplasm. But the dividing cell has its greatest increase before metakinesis. It is possible to recognize between the separating daughter chromosomes in fixed preparations thick granular protoplasmic filaments which appear after the chromosomes arrive at the poles, and then disappear. These filaments originate always at the places where the daughter chromosomes begin to separate. One must assume that just here is the region of a very intensive growth or production of protoplasm. Through a considerable growth in length of these plasma fibers, the daughter chromosomes may be pushed to the poles. This growth shows a certain direction, which is parallel with that of the spindle fibers as they appear in fixed preparations.

The movement of the chiomosomes proceeds always in the direction of the achromatic fibers. Even when the fibers do not appear *in vivo*, it must be assumed that the substance of the spindle is not isotropic. There are at least some conditions present which lead to a fixation of the spindle substance in a certain orientation. The same condition also might cause the orientation of the growth of protoplasm between the daughter chromosomes. On the basis of this explanation it is also possible to comprehend why in the first heterotypic division the two chromosomes of a pair lie parallel to each other. Only in this way is it possible that a plasma fiber growing between the two chromosomes could push them to the poles of the figure.

That the direction of spindle fibers corresponds with the direction of the movement of chromosomes is to be seen from the fact that, in spindles with irregularly oriented fibers, chromosomes also move in various directions. That is the case with some polycentric figures. It is easily possible to produce such figures in normal vegetative tissues under the influence of benzol vapors. Twenty-five years ago I undertook such experiments and they gave surprising results. In root tips of *Pisum* and *Vicia* which were exposed for 1 or 2 hours to the influence of benzol vapor, irregular polycentric figures appeared. In consequence, the chromosomes moved irregularly toward several poles. Instead of two daughter nuclei, several nuclei, generally of different size, originated. Often single nuclei were formed containing single chromosomes, and dumb-bell-shaped or even ring-shaped nuclei appeared. It is to be noted that these processes differ from those in which chromosomes are transported, after the destruction of the achromatic figure, in an entirely irregular manner, as, for example, after a repeated chloralization, or after being subjected to the influence of carbon dioxide.

I do not wish to discuss here the question whether the spindle fibers really exist or not *in vivo*. They are not to be seen in microphotographs of living pollen, or in spore mother cells. All experiments prove that they are formed of a special substance. They may appear *de novo* in every mitotic division. That the achromatic figure consists of a special substance is variously supported. In the first place, it differs microchemically from the other cytoplasm. Then, again, it does not mix, during mitosis, with the other cytoplasm and can exist without any connection with the nuclei. Sometimes the daughter nuclei are torn by the centrifugal force from the phragmoplast; nevertheless the latter preserves its shape and persists for a relatively long time as an independent structure.

If we treat dividing cells with nicotine, cytokinesis stops and the daughter nuclei separate from the phragmoplast. The latter may exist for several hours as a barrel-shaped structure in the cell.

Under the influence of chloroform the achromatic figure degenerates quickly and in its place appears a homogeneous or granular substance, which sometimes has the appearance of nucleoli. Still more striking is this process in meristematic cells placed for some time in a solution of cobalt chloride. The achromatic figure changes to granules which flow together in a manner similar to the behavior under the influence of chloroform. In resting cells such masses also appear, so that they are to be seen in nearly every meristematic cell. Since in dividing cells these masses originate through the transformation of achromatic fibers, it can be assumed that this substance is present even in resting cells, though not in a fibrillar form. This fact supports the theory of a special kinoplasmic substance.

It is possible that this substance is a constantly present constituent of the cell; it appears during mitosis as the achromatic spindle, and remains in reserve through the resting stage.

No one can deny the real existence of the achromatic spindle, especially after the microdissection experiments of Chambers. Experiments with nicotine also show that it is not an artifact, as has been supposed by A. Fischer. We must assume that the spindle exists *in vivo* as a morphologically distinct structure. As I have remarked already, the differentiation of the spindle may be brought about in connection with the necessity of dividing a certain substance between the daughter cells. Its physiological significance is uncertain.

In centrifuged cells I observed that the mitotic figure as a whole is translocated in the cell as a whole. This has been shown also by Mottier for the hairs of *Tradescantia*. When one pole of the figure touches the cell wall, the figure can easily be turned to one side until it lies parallel with the apocentric cell wall. In 1915 I described it by saying that the figure is a rigid system which passes over in such experiments from a condition of labile equilibrium to that of stable equilibrium. A fluid system never could show what we see here.

A stronger centrifugal force may deform the spindle in such manner that the fibers become curved, in the form of an S. Thus the fibers can be considered as plastic. During the prophases the nucleus can be replaced within the spindle by centrifugal force. We may conclude that at the beginning of mitosis the inner substance of the spindle is fluid. After the chromosomes have arranged themselves in the equatorial plane the whole mitotic figure becomes rigid. It was not possible to separate experimentally the single chromosomes from the spindle. But sometimes, under the influence of centrifugal force, it happened that the daughter nuclei were broken from the phragmoplast. The phragmoplast did not disappear.

When a very strong centrifugal force acts upon the meristematic cells their protoplasm may be injured. I suppose the injury to be caused by a translocation of the submicronic particles in the plasma, or generally by a change of the colloidal structure. The achromatic figure becomes granulated, the spindle disappears, and the mitotic figure appears no longer as a rigid whole.

We can not explain the true significance of the spindle, but we must conclude that it is a symptom of a normal condition in the cell. It can be observed that the chromosomes are imbedded in the substance of the spindle, or they cohere with it and are thus held together.

Chambers concludes from his microdissection experiments that the chromosomes are not fluid but rigid and elastic. After treating the meristematic cells with carbon dioxide, the achromatic spindle disappears and the isolated chromosomes may be pushed to any position in the cell without becoming spherical. From the swelling of the chromosomes in hot water it may be concluded that they represent a gel.

After the action of benzine vapor the chromosomes become shorter and thicker than they are under normal conditions. On the contrary, under the influence of carbon dioxide chromosomes become longer and thinner. Moreover, the achromatic figure disappears without any trace, the chromosomes become scattered irregularly in the cell, and they are often replaced by vacuoles. A physical explanation of the change of shape of chromosomes is very difficult if they are not fluid.

Rarely, under the influence of centrifugal force, the chromosomes become spherical. Evidently in such cases the cell has been deeply injured by the centrifugal force, and this feature has more of a pathological or necrobiotic character.

It is impossible to give a general scheme for cytokinesis. There are different types, and it is necessary to assume different mechanisms. I shall here consider only the typical vegetative cell division in vascular plants.

The process of cytokinesis, being the consequence of mitosis, is closely connected with the latter, but cytokinesis does not presuppose a real increase in the number of nuclei. Cytokinesis is rather a consequence of the separation of the daughter chromosomes. Very often under the influence of injurious factors certain daughter chromosomes fail to pull apart, leaving a chromatic bridge between the daughter nuclei. In such cases dumb-bell-shaped or amitosis-like nuclei result. Nevertheless, a phragmoplast appears and a cell wall is formed, though the latter remains incomplete. The phragmoplast extends laterally and cell-wall formation stops as soon as the new wall meets the cell wall of the mother cell.

I have studied the formation of the cell wall *in vivo* in the rhizoids of some mosses. The cell wall appears suddenly in the phragmoplast and gives the impression of a crystallization. This would be in agreement with the results of the new Roentgenographic investigations concerning the cell wall. Later on the wall extends laterally more slowly. This lateral extension of the cell wall may cease before the cell is completely divided. After repeatedly treating meristematic tissues with chloral hydrate, many cells with two nuclei appear. They always divide simultaneously. But very often the two division figures are not oriented in a parallel fashion. In this case, then, the two new cell walls are inclined to each other at a certain angle, and at the moment when their borders meet they cease growth immediately, though the cell is not yet completely divided. This occurrence also may be explained by the supposition that the formation of the cell wall is a process of crystallization.

In a multicellular organ, division proceeds rhythmically in correspondence with the intensity of growth. Therefore, the size of the meristematic cells of a certain tissue remains nearly constant. The direction of cell division is related to the inner structure of the organ and to its external shape. In physiologically equal cells the size of the cells depends upon the number of chromosomes in their nuclei. Although this dependence is not always direct, it may not be denied, and in vascular plants especially it is of general occurrence.

In many plants, through the fusion of nuclei, polyploid nuclei and cells may be produced, their size being correlated with the number of the chromosomes. The organ consists now of cells of different sizes, and nevertheless the typical form and size of the organ persist. It does not increase in size so long as the number of polyploid cells is less than half of the total number of cells. The mosaic polyploid organ, consisting of a mixture of cells, shows a normal growth. This fact indicates that for the external shape and size of organs the method of growth of the cells and the number of parts (cells) into which the protoplasm is divided are not of chief significance. It is not the cell division but rather the splitting of the chromosomes which is directly connected with the growth of the protoplasm. The chromosomes may be aggregated into groups of various sizes.

It cannot be denied that the orientation of the cell walls is of great importance for the inner structure of the organ. The more regularly they are placed, the more regular is the inner anatomical structure of the organ. With the growth of the organ are correlated changes in the orientation of divisions. It is possible to call these relations, of symmetry and inner structure of the organ, polarity. I am in agreement with Harper in the statement that in the presence of polarity and the various symmetry relations we have a fundamental distinction between the cell organization and that of polyphasic colloidal systems as they are commonly produced *in vitro*. But polarity is a characteristic of the organ as a whole no less than of single cells.

As mentioned above, it is possible to produce organs which are composed of cells of very different sizes, yet they grow in a normal way and maintain their normal external shape. The protoplasm of all neighboring cells is connected by plasmodesmen, and the whole possesses a symmetry relation of its own which does not depend directly upon the size and number of the cells of the organ concerned.

The cells maintain their polarity very strongly, and in the higher plants it is very difficult to reverse this polarity artificially or to annihilate it. Some external factors transform the usually bipolar mitotic figures into multipolar ones. Such multipolarity sometimes may occur in highly polyploid cells; but the normal polarity of the cell is not destroyed by such divisions. The multipolarity of the mitotic figure leads in such cases to a reduction of the number of chromosomes. But the mode and mechanism of this reduction differ completely from that of a reduction by means of two meiotic divisions.

It is impossible to give at present a complete explanation of the mechanism of mitotic division. It is a very complex phenomenon, and only further experimental investigations can bring us to a real solution of the problem.

PROTOPLASMIC STRUCTURE[1]

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Introduction

Protoplasm is a heterogeneous, organized, aqueous dispersion of crystalloids, proteins, and fatty substances. The structure of protoplasm is the structure of such a system. The crystalloids are, for the most part, in true solution. The fats exist in the dispersed state of an emulsion. The physical state of the proteins is not with certainty known. Experimental work and theoretical analysis point to a fibrous structure for protein jellies, an interlacing mass of slender, crystalline fibers of molecular, colloidal, or, possibly, of microscopic dimensions. Such a structural framework permeated by a solution of crystalloids and an emulsion of fats is the structure of living matter.

Protoplasm, regarded as a mixture of salts, saccharides, proteins, and fats is protoplasm in the broadest sense of the term. If, however, we restrict our definition of protoplasm to living matter alone, then we are confronted with the task of deciding what is living and what not. To some, the large percentage of water in protoplasm is alive because it is part of a living system. So also is a droplet of oil and a globule of yolk in a cell alive. A second view-point is one in which the solution of crystalline substances and the emulsion of fatty material, especially the microscopically visible emulsion in protoplasm, are regarded as essentially non-living matter, as food, while the protein content represents protoplasm in its simplest form, the ultimate living substance itself. Whatever our conception of protoplasm may be, we are forced to grant the presence of some extraordinary substance or mixture of substances which forms the basis of living matter. Undoubtedly this is protein in character, probably of the same general chemical nature as the enzymes.

Protoplasm, in the broad, generally accepted concept of the term, consists of three fundamentally different types of systems, intimately mixed, functioning in unison, each dependent upon the other—a lyophilic protein complex bathed by solutions of salts and sugars and emulsions of fatty substances.

Protoplasmic Emulsions

The visible emulsion. Protoplasm viewed through the microscope presents the picture of a typical emulsion. The particles of this visible emulsion vary in size from mere specks, which are probably liquid in nature, to globules 10μ

[1] Presented before the International Congress of Plant Sciences, Section of Cytology, Ithaca, New York, Aug. 17, 1926.

or more in diameter. This protoplasmic emulsion may assume a variety of forms. Usually it is a conglomerate of liquid particles differing in size, arrangement, and chemical nature. In ova, the dispersed phase of the emulsion is yolk and the globules are fairly uniform in size. Among the larger liquid particles of ova are tiny "microsomes." Globules intermediate in size between macrosomes and microsomes exist, but the mixture presents a moderately uniform appearance of large and small dispersed droplets.

In other types of protoplasts the emulsion is quite heterogeneous. This is true in myxomycetes. In parts of still other protoplasts, such as the ectoplasm of certain protozoa, the visible protoplasmic emulsion is very uniform in texture, assuming an almost perfect symmetry. We obtain here, in the ectosarc of the ciliate *Euplotes* for example, typical alveolar protoplasm as described by Bütschli ('94). The alveoli are 2μ in diameter and of nearly exact hexagonal, occasionally pentagonal, shape in optical section. In geometrical form the alveoli are probably rhombic dodecahedrons.

The alveolar structure of protoplasm in the strict Bütschlian sense has been unjustly condemned as an "artifact" (Hardy '00), as a "post mortem process" (Gaidukov '10). This is not true. The configuration, precisely as Bütschli described it, is to be found in a remarkably symmetrical form in numerous protoplasts, and as the visible structure of nuclei as well as of cytoplasm. What is not true is that the alveolar structure is of "universal" occurience and that it represents the ultimate structure of protoplasm.

The ultramicroscopic suspension. Granule-free hyaloplasm, which is optically structureless when viewed in direct light, becomes, through the ultra-microscope, a striking colloidal dispersion of myriads of scintillating, dancing particles in a liquid medium. These colloidal particles may be the disperse phase of an ultramicroscopic emulsion, the counterpart in the realm of colloidal dimensions of the microscopically visible emulsion.

The rôle of emulsions in life processes. The protoplasmic emulsions, whether microscopic or colloidal, undoubtedly play an important part in vital phenomena, but they probably are not the seat of those physiological processes which distinguish living matter from non-living, and certainly not the basis of those physical properties which distinguish that group of (lyophilic) colloidal substances, of which protoplasm is one, from other (lyophobic) types of colloidal systems. Imbibition, coagulation, rigidity, and elasticity are properties which the physical constitution of emulsions precludes; on the other hand, these proper, ties are common to the proteinaceous lyophilic colloids to which gelatine, albumin, and protoplasm belong.

Properties of the Protoplasmic Jelly

The colloidal state of protoplasm. The term "jelly" is here used in reference to protoplasm because the physical properties of the living substance are properties of jellies. But if the term "jelly" connotes to the reader a substance always firm enough to hold its shape unsupported, then the word is, in part, misleading, since protoplasm is most often liquid enough to flow and frequently

quite thin, though never watery—in this sense, therefore, a sol. The term "sol," however, is less accurate than "jelly" as an indicator of the colloidal state of protoplasm, since we do not at all associate with sols those properties which characterize living matter, namely, imbibition and elasticity. The difficulty lies in the absence of any very definite criterion of the sol or gel state. The nearest approach to such a criterion is Brownian movement, but it is inaccurate as will be later shown. It really matters little what we call protoplasm, whether sol or gel, so long as we fully appreciate the fundamental properties which establish its identity as a colloidal type.

It is the purpose of this paper to consider, in addition to structure, only two of the physical properties of protoplasm, namely, elasticity and viscosity, the first of which as much as any other serves as an indicator of protoplasmic structure.

The elastic property of protoplasm in general. That protoplasm is elastic was undoubtedly suspected by the earliest workers on living matter, as is indicated by their descriptions of it as a "viscid mass" (von Mohl '46), a "glutinous jelly" (Dujardin '35). No experimental evidence of the elasticity of protoplasm seems to have been presented until the tensile strength determinations made by Pfeffer ('90). Even these experiments did not have to do directly with elasticity but with cohesive power, as Pfeffer called it. Investigators of the last half century have made indirect observations which clearly point to marked elastic properties of protoplasm. Especially evident as an indicator of protoplasmic elasticity is the stretching of the fine protoplasmic strands which connect a plasmolyzed protoplast to the cell wall as plasmolysis proceeds. Other similar contractility phenomena must have constantly emphasized to the earlier students the ever-present property of elasticity in protoplasm. But newer methods have made possible not only a more convincing visual demonstration of the elasticity of protoplasm but also the actual measuring of it.

Stretching protoplasm between microneedles, and attracting, by means of an electro-magnet, a metal particle embedded in the living substance, permit relatively precise measurements of protoplasmic elasticity. Some of this work has been published (Seifriz '24, '26). Additional facts pertaining to myxomycete plasmodia are presented on the following pages.

Micrurgical[2] *evidence.* It is no difficult task to stretch protoplasm between glass needles with the aid of a micromanipulator (Péterfi '23, Seifriz '26). Two exceedingly fine needles (tips not over 1–2μ in thickness) are inserted into the living material which is suspended in a water film on the under side of a glass slip which serves as the cover of a moist-chamber under the microscope objective. The microneedles are brought up into the protoplasm in close proximity to each other and then separated. Protoplasm may be stretched in this manner until, in some cases, it is a barely visible thread. Its capacity to return to its original size when the needles are removed varies greatly with different types of protoplasm and with the physical state of the protoplasmic mass.

[2] Micrurgy (*micros*, small; *ergon*, work), a word coined by Péterfi ('23) to denote microscopic operations.

Liquid protoplasm of low viscosity may be stretched but very short distances. Protoplasm of moderately high consistency may be stretched to extraordinary lengths. Firm protoplasm of high viscosity is very resilient (elastic) but its stretching limit is correspondingly low. Coagulated protoplasm is either poorly elastic or wholly inelastic. In the latter case it is a soft plastic gel. All of these degrees of elasticity have been demonstrated in the protoplasm of Myxomycetes and of red blood cells (Seifriz '26) by micrurgical methods with the aid of a Péterfi (Zeiss) micromanipulator.

Erythrocytes. The membrane of blood corpuscles is, at times, moderately elastic, tending more toward a plastic condition, and at other times highly elastic and resilient. The amphibian (*Amphiuma*) erythrocyte may be stretched to three times its original length. Of extraordinarily high stretching capacity is the nucleus of an amphibian corpuscle. A nucleus isolated from the cell by dissection may be stretched from an original diameter of 14μ to a slender double thread 350μ in length, and on removal of the needles the nucleoplasm returns to nearly its original proportions.

Plasmodia. The myxomycete worked upon in these studies was chiefly *Badhamia* growing in culture on *Stereum*. It is impossible by any known method to measure accurately the degree of elasticity or viscosity of flowing protoplasm. These properties of protoplasm in motion can only be estimated by indirect observations. If a plasmodium is torn by microneedles at a point of rapid streaming (over 200μ a second) the protoplasm will flow out with the ease and speed of a moderately viscous substance (specific viscosity estimated at 20 to 30 times that of water). If, now, this same flowing protoplasm of rather low consistency is prevented from streaming by pressure with a needle against the plasmodium across the capillary, and the now quiescent protoplasm is immediately dissected, the latter is found to be highly viscous, in some instances plastic and poorly elastic, in others, very elastic. It is thus seen that the same mass of protoplasm may change quickly in its viscous properties, from a low value when streaming to a high value when quiet, and may vary considerably in its elasticity.

The low viscosity value (9 to 18 times that of water) obtained by Heilbronn ('22) for the endoplasm of slime-moulds is undoubtedly correct and in close agreement with the values given here, but it represents only one value of many, throughout a wide range, which protoplasm may assume. The protoplasm which flows out of a tear made in a plasmodium often closely resembles bread-dough in its physical properties, highly viscous, slightly plastic, and extremely elastic. Globules of exuded protoplasm frequently form on the surfaces of plasmodia. The protoplasm of these is usually very elastic and may be stretched into fine threads 650μ in length. We are dealing here with a rather firm, resilient and elastic jelly.

In these highly viscous elastic globules Brownian movement of minute particles goes on. This is possible only on the assumption that the protoplasmic mass consists of a structural framework in the interstices of which particles carry on their kinetic motion suspended in a matrix of lower consistency than the mass as a whole. The presence of Brownian movement in a highly viscous

elastic jelly points to two fundamental facts: first, Brownian movement is not a reliable criterion of colloidal state in heterogeneous systems, and second, protoplasm consists of two types of matter, one a liquid of relatively low consistency permeating a framework to which living matter owes its elastic properties. Micrurgical dissections clearly support this. Frequently a very clear picture is obtained, in protoplasm under dissection, of two substances, a thin liquid of apparently even texture permeating a distinctly "stringy" substance.

The elastic properties of protoplasm may be demonstrated and measured, as previously indicated, by inserting a metal particle (preferably nickel) into the living substance and attracting the particle with an electro-magnet. The distance the particle moves toward the magnet, provided it returns to its original position on release of the current, is a measure of the stretching capacity and therefore the elasticity of the protoplasm. The method was devised by Alfred Heilbronn ('22) for viscosity determinations, and independently by Freundlich and Seifriz ('23) primarily for elasticity measurements of non-living and living colloidal systems. The two latter authors incorporated the use of a micromanipulator into their technique, thus permitting the handling of more minute (7μ) metal particles.

Considerable difficulty is involved in obtaining measurements of elasticity of the protoplasm in the tiny capillaries which ramify through the flat fan-shaped advancing processes of a plasmodium. The fate of one particle may be described. An 8μ nickel particle was inserted into a plasmodium; ultimately, it was carried along with the streaming protoplasm in a capillary until it became lodged in the ectoplasm. At this moment the magnetic field was applied (at right angles to the capillary) and the particle was drawn out of the ectoplasm across the slowly flowing protoplasm in the capillary and embedded in the ectoplasm on the opposite side. The rate of travel across the capillary was not very rapid, indicating a consistency of the endoplasm distinctly not watery.

The most precise measurement of the value of Δ, the maximum stretching capacity, that is, the greatest distance a metal particle moves and yet returns to its original position on the release of the magnetic attraction, obtainable for the protoplasm filling a capillary gave a value of $\Delta = 4.4\mu$ with an 8μ nickel particle. This is not a high value, nor can it be regarded as precise; it merely indicates that the thinner active protoplasm filling the capillary is elastic.

When globules of exuded protoplasm are spontaneously formed on the surface of a plasmodium, or when a mass of escaped protoplasm is obtained by an outflow through a tear, a microscopic nickel particle may be readily inserted and attracted by the electro-magnet. Maximum stretching values then obtained equal 292μ, indicating a highly viscous, tenacious jelly of great stretching capacity. A value of 292μ for Δ (maximum stretching capacity) approximates that obtained by Freundlich and Seifriz ('23) for egg albumin, and greatly exceeds the maximum values for gelatin.

The ectoplasm of plasmodia becomes dilute at the surface of an advancing pseudopodium, and then exhibits properties similar to the active endoplasm.

When quiescent the ectoplasm is a firm elastic jelly which can be stretched but slightly.

Thin, active, streaming endoplasm as found in the capillaries may, when it is inactive, become as firm a jelly as the quiescent ectoplasm. There is no essential difference between ecto- and endoplasm so far as their physical properties go, except that the former is usually quiescent and firm and resilient, while the latter is more often thin, of low consistency, and poorly elastic. Either may assume the usual physical state of the other.

Criticism is sometimes made of the possible injurious effect of micrurgical operations on living protoplasm and a resulting departure from the normal physical state of the protoplasm. It is true that a puncture or tear in protoplasm may bring on pathological changes, but these are immediately detectable. One would expect that the insertion of a 30μ particle into a plasmodium, especially when done by hand-operated instruments, as in the case of Alfred Heilbronn's ('22) experiments, would cause serious injury, but Heilbronn guarded against this by allowing the plasmodium itself to form a protoplasmic mass over the particles before his measurements were made. The particle was, therefore, very probably imbedded in normal protoplasm. It is less likely that the insertion of more minute particles handled on the tips of delicate microneedles, mechanically controlled, would cause pathological changes in protoplasm. The fact that often these metal particles are later carried in the stream of the actively flowing protoplasm is conclusive evidence that the protoplasm, if it suffered injury at the time of insertion, has recovered to normal activity. A tear in a plasmodium likewise causes but temporary injury. The protoplasm which escapes through a tear in a plasmodium may remain fully active and apparently quite normal, except for a discarded bit where injury was too severe for recovery. To cite one instance: a large tear was made in an active capillary of a plasmodium, the streaming trotoplasm flowing out to form a large irregular globule adjoining the capillary. The protoplasm in this escaped mass resumed streaming back into the main stream of the capillary for fully five minutes, until the volume of the escaped protoplasm was greatly reduced, leaving isolated a small area of degenerate protoplasm. If injury is severe, the protoplasm coagulates. If injury is mild, the protoplasm remains alive and active.

There are those who are greatly disturbed over any evidence which points to a high consistency of protoplasm. Their difficulty lies in their inability to appreciate that protoplasm is a true lyophilic colloid capable of forming an elastic, turgescible jelly, and as such assumes an infinite number of degrees of viscosity, from a very thin liquid ("sol") to a firm jelly. Evidence of the firm state of protoplasm is so great that the following would appear superflous if it did not emphasize the complete absence of any liquid protoplasm in regions of the protoplast which were only shortly before actively streaming.

The broad, often fan-shaped advancing pseudopodia of myxomycetes are, when well developed, replete with capillaries of actively flowing protoplasm. Activity may, at times, cease over large areas, while continuing elsewhere. If the quiescent regions are explored with microneedles shortly after streaming

has stopped, often no liquid *protoplasm* can be found. Either one must make the absurd assumption that no endoplasm is present—since ectoplasm is by all agreed to be usually of high consistency—or grant what is true, that the previously liquid endoplasm has become firm while inactive.

With these changes in viscosity there usually occur similar changes in elasticity. However, viscosity is not always an accurate indicator of elastic value. It has been stated that highly viscous protoplasm may be poorly elastic while other protoplasm may assume the same degree of consistency and yet be highly elastic. What the difference in elastic value of the two masses of protoplasm of like consistency is chemically due to cannot be said. Similar differences in elasticity have been observed in two samples of soap which were presumably of the same chemical constitution ('25). As for protoplasm, we may evade a direct answer to the question "Why is the living substance more elastic at one time than at another, or in one protoplast than in another, even though of the same consistency?" by saying that the physiological state of the two protoplasts or regions differs.

The chemical difference which is responsible for different degrees of elasticity at the same viscosity value is unknown; the physical factor is, we believe, definitely known. The cited case of the two soaps (Seifriz '25) and many other observations point to a definite type of structure for elastic lyophilic colloids which is lacking in inelastic colloidal systems.

The Structure of Jellies

There is much evidence in chemical literature on the structure of non-living elastic jellies which clearly suggests a fibrous type of structure. We shall here consider one experimental fact which is in line with other experiments and deductions in this field. Of two samples of presumably the same soap, namely, sodium stearate, received from different manufacturers, one proved to be inelastic and one elastic (Seifriz '25). Examined under the microscope the elastic soap possessed rod-shaped dispersed particles, while the dispersed microscopic particles of the inelastic soap were spherical in general outline. Further, the elastic soap built up crystalline masses of fibrous structure; this the inelastic soap lacked the capacity to do. The highly elastic ammonium oleate soap (Seifriz '25) forms an extraordinary structure of an entangled mass of very long intertwining threads.

Two prerequisites seem to be necessary for a colloidal system to be elastic: first, the structural unit must be fibrous, a crystalline rod or a thread; and second, the system must possess the capacity to build up an entanglement, a "brush-heap" as the chemists have graphically termed it, of the fibrous rods or threads. A system with spherites as structural units, as, for example, the inelastic sodium stearate soap, or an emulsion of two liquids, or, as a crude example, sand, cannot be elastic. Your brush-heap is springy, your sand-pile not.

Having optically demonstrated the type of structure in elastic and inelastic soaps, and having experimentally demonstrated elastic properties in protoplasm, the conclusion as to the structure of protoplasm is obvious.

The dimensions of the fibers which build up the elastic framework of protoplasm are not known, nor are they with certainty known in most jellies. Wilson, Loeb, and Langmuir believe the unit of structure of gelatine to be the molecule, or a chain of molecules. Procter apparently regards it as possible that these linear molecules may be aggregated into larger (colloidal) structural units. Others believe that the fibers may assume microscopic dimensions, which seems proven by photographs (Seifriz '25) of them in elastic soap curds.

That the fibers of which protoplasm is built may, at least at times, assume microscopic dimensions is suggested by the "stringy" appearance of myxomycete plasmodia when subjected to stretching by microneedles, by the frequent appearance of a fibrous structure in fixed and stained protoplasm, by the presence of spindle fibers, and of nerve and muscle fibers.

Conclusion

Whether or not we can say that the fibrous structure of protoplasm has been conclusively proven is, of course, a matter of personal opinion. Those there are who will oppose this theory, others feel convinced that it is true. But this much is certain, that on the basis of a fibrous structure, an interlacing mass of tenuous, probably crystalline fibers, the elastic properties and the imbibing powers of protoplasm are intelligible. No other known structure will serve to explain the mechanics of the physical properties of the living and of non-living jellies.

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ON THE NUMBER OF CHROMOSOMES IN THE TWO DIFFERENT FORMS OF ECTOCARPUS VIRESCENS THURET[1]

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Since the discovery of the alternation of generations in the family of the Laminariaceae, the classification of the brown algae has taken a great stride forward and the relationship between the considerably varying sub-groups is now much more evident than formerly. To this result the demonstration of the cytological conditions in many brown algae, especially the place of the reduction division in the life cycle, has been highly conducive. Thus we are now aware that a reduction division in the Laminariaceae occurs in the sporangium before the formation of the zoospores, this having been conclusively shown by Kylin in 1918 in the case of *Chorda Filum* (L.) Stackh.

It was thereby demonstrated that the place of the reduction division in this family is exactly the same as in the family of Cutleriaceae where the reduction division was demonstrated by Yamanouchi in *Cutleria* (1909) and *Zanardinia* (1911, 1913). In these it was found to occur at the first division in the unilocular sporangia. Through the work of Williams and of Mottier it has long been known that the place of the reduction division in *Dictyota* is likewise in the unilocular tetrasporangium. Finally, there was published a few years ago (1923) some very careful work by Margery Knight on the "Life-history and cytology of *Pylaiella litoralis*" which greatly extended our knowledge of the development and cytology of the lower Ectocarpaceae, showing at the same time with all clearness that the course of development in the lower Ectocarpaceae may vary a good deal and that, while a regular alternation of generations occurs, it does not proceed so regularly or so conformably to law as in the more highly organized plants, this being due to the fact that each of the various generations has the power of regenerating the same generation again. In *Pylaiella* also the reduction division takes place in the unilocular sporangia. The condition described may consequently be regarded as the general rule in the whole of the great group Phaeophyceae.

For the better comprehension of the problem with *Ectocarpus virescens* I desire briefly to review the results of Miss Knight with regard to *Pylaiella.* This alga occurs in a haploid monoecious sexual generation which forms isogametes in plurilocular sporangia, or so-called gametangia, of the type normal for the Ectocarpaceae. This haploid generation thus forms haploid gametes from whose fusion products the diploid plants are developed. These diploid *Pylaiella* plants

[1] Presented before the International Congress of Plant Sciences, Section of Cytology, Ithaca, New York, Aug. 17, 1926.

are morphologically similar to the haploids, but sooner or later they bear unilocular sporangia in which the reduction division is at the first mitosis, so that the zoospores are haploid. These zoospores now form on germination haploid *Pylaiella* individuals again. This development is thus, broadly speaking, in agreement with the *Dictyota* type, with the single exception that the haploid sexual generation is monoecious and isogamous.

The development does not, however, always run so regularly. The liberated asexual spores give rise in the early spring to haploid plants. They produce from plurilocular sporangia (=gametangia) haploid gametes from whose fusion products diploid plants are developed. The latter reproduce themselves, however, by means of diploid neutral spores developed from diploid plurilocular sporangia of the same appearance as the haploid plurilocular ones. These diploid spores are neutral and develop without any sexual fusion, and they may give rise to a long succession of diploid plants during the early summer, but later on the cycle of development is completed by the production of unilocular sporangia in which the reduction division takes place.

The haploid individuals are also able to propagate haploid individuals again by the parthenogenetic development of the swarm spores from the plurilocular organs. If, as sometimes happens, unilocular and plurilocular sporangia occur on the same individual, one may certainly infer that these must be of diploid nature.

With our knowledge of this course of development we might dare to assert that in the Ectocarpaceae the plurilocular sporangia must be deemed the more original and primitive organs of reproduction than the unilocular, in which latter the reduction division appears. The unilocular sporangia are a somewhat later addition, appearing in and with the diploid organization, since they occur only on the diploid generation, never on the haploid. The fact that the plurilocular organs give rise to both sexual gametes and sexless zoospores, so that the potential gametes can also develop parthenogenetically, must be regarded as primitive. Non-sexual propagation in plants must undoubtedly be deemed more primitive than sexual. On this assumption, it may be stated for the family of the Ectocarpaceae that plurilocular sporangia which yield haploid zoospores, germinating directly, are the most primitive form of the organs of reproduction. One may then regard a sexual fusion of these zoospores as a secondary means of propagation, finally taking an established place in this group. Sexual fusion has given rise to diploid individuals which can propagate themselves by producing diploid individuals again from the diploid plurilocular sporangia, but sooner or later the unilocular sporangia are developed in which a reduction division may occur, and so haploid individuals may arise again. It seems to me indubitable that this must really be considered the simplest and most natural course of development in this family—a family which is universally looked upon as one of the most simply organized in all the great province of the brown algae.

However, there now arise a whole lot of further questions to answer and problems to solve; for it has long been known that a number of the otherwise simply organized Ectocarpaceae, unlike *Pylaiella* and the majority of the *Ectocarpus*

species, have not only one kind of plurilocular organs but several. A number of species have two and some species no less than three different kinds of such plurilocular organs. *E. secundus*, for instance, has two kinds of plurilocular sporangia. The one kind has numerous small loculi, the other but a few large loculi. The explanation of these different types is a simple one; there is here a sexual morphological differentiation into male and female organs, and several investigators have shown that the swarm spores of different sizes formed in the different organs do not fuse with each other but that there is fusion only of the small ones with the large ones. These plurilocular organs may thus be properly designated as oogonia and antheridia. In *Ectocarpus secundus* (=*Giffordia*) Sauvageau has likewise noticed that at certain times the antheridia disappear, that is, the individuals which bear plurilocular gametangia with small loculi; but oogonia continue to be formed, the eggs being now developed without fertilization. On the other hand, the investigations of Sauvageau have demonstrated that the contents of the antheridia, the male gametes, are not capable of parthenogenetical development. They are so small that their store of nutrient is probably too little for this. Thus we see that even with morphological differentiation into oogonia and antheridia the transition to parthenogenetical development is very easy, at any rate in the case of the female gametes. This type having two kinds of plurilocular organs, is thus easily explained. Those types, on the other hand, which have not less than three different kinds of plurilocular sporangia are more difficult to explain. Sauvageau found in *E. Padinae* three morphologically different types of plurilocular organs: one kind with very small loculi which he, no doubt correctly, designates antheridia, and another kind with middle-sized loculi which he designates meiosporangia, and finally another kind with rather large loculi which he designates megasporangia. Which of the two last-mentioned kinds should be designated oogonia is not known, and respecting the nature and function of the third remaining kind we are still completely ignorant. In the light of Margery Knight's investigations of *Pylaiella*, from which it appears that *Pylaiella* has both haploid and diploid plurilocular sporangia (though they are morphologically alike), we are, however, tempted to think that one or other kind in *E. Padinae*, either meio- or megasporangia, produce haploid gametes, which may be termed eggs, and then, perhaps, the other kind may be diploid with spores which are developed without fertilization. In contrast to *Pylaiella* they would, in *E. Padinae*, be morphologically somewhat unlike the sexual spores (=gametes) and thereby the three different kinds of plurilocular organs would receive a natural explanation.

Accordingly there may be the following kinds of organs, all slightly different: (1) antheridia with male gametes; (2) oogonia with female gametes; and (3) neutral zoosporangia producing spores which always develop without fusion. It should, however, be added that, if this interpretation is correct, the unilocular organs should also be found in which the reduction division takes place. Yet another *Ectocarpus* with different kinds of plurilocular organs is *E. virescens* Th., known to and described by the great French algologist Thuret. It is of this species I now intend to speak after this rather lengthy introduction.

Ectocarpus virescens has been the subject of a thorough life-history investigation by the French algologist Sauvageau at Bordeaux, and it is from him I have obtained the material for this cytological investigation. The alga grows in the Atlantic on the shores of England and France.

E. virescens is not known with unilocular sporangia but has regularly plurilocular sporangia of two different kinds. One has numerous small loculi, so-called meiosporangia, corresponding in form and appearance with the plurilocular organs occuring in the bulk of *Ectocarpus* species, for example, *E. siliculosus.* The other kind, megasporangia, are as big as meiosporangia with fewer but larger loculi, and in them swarm cells or zoospores are formed which are considerably larger but likewise mobile and furnished with cilia exactly like those from the meiosporangia. In spite of the most thorough investigations Sauvageau never succeeded in observing these organs on the same individual either at the same or at different times. It is thus clear that there are two kinds of individuals in *E. virescens*, those with meiosporangia and those with megasporangia. Forms with meiosporangia are most common. The meiosporangia are about 100–115μ long (90–155), 20–24μ wide, and the loculi are 6–7μ in depth. The zoospores are 16–23μ long and 6–8μ wide and always have a small red eye spot. What, then, is the relation between the zoospores which are formed in these organs? Sauvageau has found that they do not fuse together; they are consequently not gametes, but are developed directly as neutral spores. The form with megasporangia is rather rare and clearly confined to definite localities. The megasporangia are 100–150μ long and 20–30μ wide and the zoospores are usually formed in two rows only along the whole length of the sporangium. The megaspores are about 26μ long and about 13μ wide, that is, about twice as wide as the meiospores. The megaspores have no eye spot. The megaspores are not very actively mobile and often even germinate inside the megasporangium. They all germinate directly without any fusion with each other. Neither do zoospores from different individuals conjugate with each other. Lastly, it may be mentioned that Sauvageau also investigated the possibility of mega- and meiospores conjugating with each other, but in every case he reached a negative result. The different kinds were always developed directly, each by itself, without any previous conjugation. As both meio- and megaspores germinate directly into definite, new *Ectocarpus* individuals, the possibility is thereby excluded that any unilocular sporangia should conceivably be able to occur on any kind of individuals which stood in the relation of an alternating generation to individuals with plurilocular organs, whose affinity with them was still unknown.

Thus we stand here face to face with an unsolved riddle, namely, what are the functions of the different kinds of sporangia and spores in *E. virescens*, and what can be the cause of this remarkable dimorphism if it is not a sexual differentiation? The idea has arisen that one of the two kinds of zoospores may, after all, be gametes, in which case it would more likely be the meiospores. With regard to *Pylaiella*, Knight states that the spores which are formed from the plurilocular sporangia and which fuse are always haploid. Those which do not fuse are either diploid (and this is the case with the majority of them) or

sometimes even haploid, in which latter case they must be looked upon as facultative gametes developed apogamously. The latter type of spores evidently has a varying propensity either to fuse or to be developed apogamously and it is possible that this propensity or tendency may be influenced by external factors. This observation may perhaps justify the suspicion that, after all, a fusion of meiospores of *E. virescens* is not quite out of the question although, under the conditions in which they have hitherto been observed, they have always been developed directly, that is, without fusion. There is, however, this possibility, and then it might be conceivable that the individuals which form the megasporangia are diploid and that the morphological difference might have a cytological basis, the one group being haploid, the other diploid.

A cytological investigation of the two forms of *E. virescens* should therefore be of interest, as it would at least contribute something to the solution of the problem.

That was, indeed, the reason why I had sent to me by Professor Sauvageau the material for the examination of the two different forms of *E. virescens*, and I shall now give an account of the result of this cytological investigation.

The meiosporangia are established as one-celled lateral branchlets from the purely vegetative branches and the former are early distinguished by their more abundant protoplasmic contents and their large nuclei. The nucleus soon passes into divisions which result in the formation of a single row of about eight cells, each one with a large nucleus and numerous chromatophores, together with numerous deeply staining bodies that may be of the nature of pyrenoids. Nuclear divisions are very rare in my material. Before the division the nucleus increases in size, the faint reticulum becomes more or less clearly marked, and the chromatin precipitates as rather sharply marked dots. If the number of such larger dots corresponds to the number of the chromosomes, this number cannot be very high, since more than quite a small number of such dots (6–7) can never be observed. Futhermore, this number agrees with the approximate number of chromosomes which I believe I have observed in the few divisions I have seen. It is fairly certain that the number of chromosomes does not exceed ten. I have never observed any indication that a reduction division takes place in the plurilocular sporangium. This is indeed most unlikely, and I mention it in this connection only because Miss Knight once thought she had found that a reduction of the number of chromosomes had occurred in a plurilocular sporangium. She says (p. 35): "In certain plants the somatic nuclei showed the double number of chromosomes, but the latest divisions of the plurilocular sporangia showed unquestionably the haploid number." There was no sign of a synapsis to be seen in the division of the mother cell of the sporangium. In my opinion the whole question is in great need of further study.

To repeat, the number of chromosomes in the meiosporangia, even though not accurately determined, seems in any case not to exceed ten.

What are the facts respecting the number of chromosomes in the megasporangia? The development and first divisions of the megasporangia are exactly similar to those of the meiosporangia except that the divisions cease earlier,

so that the number of loculi is much less. The nuclei are of exactly the same size as in the meiosporangia, and in the reticulum the chromatin dots are as numerous, or rather as few, as those in the meiosporangia. In the divisions which I have succeeded in observing the number of chromosomes is certainly not more than ten, being thus, in all probability, the very same as in the meiosporangia. Anyhow, the possibility of the number of chromosomes in the one type of sporangium being twice as many as in the other is excluded. By this means I have succeeded in showing that the two different types of *E. virescens* as regards the plurilocular sporangia (meio- and mega- forms) have no corresponding difference in the number of chromosomes and, consequently, that their relation to each other cannot be that of haploid and diploid stages, respectively. This is, moreover, confirmed by the fact that unilocular sporangia have never been met with in this species, although they must incontestably exist if real fertilization is to take place.

How, then, are the two forms to be explained? To this I am at present unable to give any definite answer, but while writing this lecture I received a letter from Professor Sauvageau saying that he had succeeded by cultures of megaspores in obtaining plants which form megaspores again. The formation of megaspores is thus a hereditary character which is transferred to the progeny. This is an important fact but does not, of course, tell us much about the real nature of these different spores.

The final explanation of the matter must therefore be postponed for the present. The explanation which, from the cytological point of view, seems to me most likely is that the differentiation may still possibly have been of sexual nature—the similar number of chromosomes favors this, or, at any rate, is not against it— but the sexuality has become eliminated so that, instead, apogamous development has become the rule for both kinds of gametes, both having lost the power of conjugation.

With our knowledge of the lability which in this respect prevails in *Pylaiella*, this is no improbable supposition. Nor is it surprising that the power of forming different kinds of sporangia is inherited by the progeny.

The idea that we have here to do with two different species or races is decisively contradicted by the evidence of experienced algologists who have studied this alga in nature. We are here confronted with an unsolved riddle. It may be that the sudy of *Ectocarpus Padinae*, with the three different kinds of plurilocular sporangia, will bring the problem nearer to a solution. In any case the study of these lower brown algae shows that their development and life cycle is not so regular as in the higher algae and, above all, that we must not be guilty of the not uncommon mistake of seeking to generalize the laws concerning the course of development which have been established for certain higher forms, and to apply these to the lower forms. This, at least, we have learned from what we now know of the development and cytology of *Ectocarpus virescens*.

CHROMOSOME STRUCTURE IN MITOSIS AND MEIOSIS[1]

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Any conception of a chromosome as a body of uniform physical constitution is obviously untenable in the light of the many reports of internal differentiation of one sort or another. Chromosome structure may be evidenced by surface contours (gross structure) and by internal differentiations as granules, threads, etc. Interpretation of internal structures is the harder task, and its importance lies in the possibility of correct analysis of the more minute longitudinal differentiation of the chromosome and of the method of chromosome division.

If made of different, physically isolated substances, then the structure of the chromosome becomes of vital interest in connection with the inheritance of specific parts of it, and if the morphological parts of it are to be specifically inherited, a physical mechanism is to be expected in the chromosome to effect this in an orderly way. Discussions of the forms of chromosomes were of uncertain value until it was shown that the point of attachment of the spindle fibers is a fixed morphological feature. Such permanency of fiber attachment has long been claimed or tacitly admitted, and demonstrations of it have not been lacking, especially for animal cells. For the chromosomes of plant cells the question did not seem a vital one until the correlation of gross chromosome structure with external characters of the plant was found to be of immediate value in genetical studies. Chromosome form will probably ultimately prove of more precise value than chromosome number in differentiating species from each other on cytological grounds.

Němec first carefully called attention to the constrictions which characterize many chromosomes, but this paper ('04) failed of the full recognition it deserved as a report on normal cell features because of its emphasis on abnormal conditions, especially the conspicuousness of the constrictions under the influence of chloral hydrate and other reagents, which distracted attention from the normality of the phenomenon. Tschernoyarow ('14) in his paper on *Najas* gave us what have become our most familiar figures of constrictions and satellites in plant chromosomes, although cases demonstrated since are even more striking. Sakamura ('20) presented a better analysis of chromosome form as a normal state, but he also refers to the influence of chloral hydrate. Sakamura worked mostly on Leguminosae and a few monocotyledonous plants. An important feature of his work was that he effected a comparison of various species of a single genus (*Vicia*), which suggested the value of this type of analysis in genetical

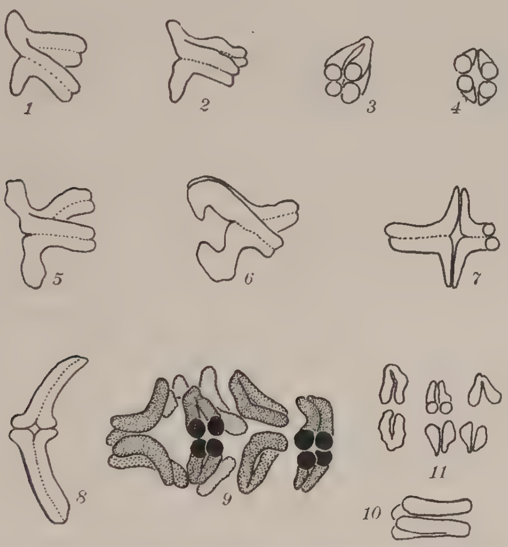
[1] Presented before the International Congress of Plant Sciences, Section of Cytology, Ithaca, New York, Aug. 17, 1926.

studies. He also demonstrated the fact that the complete series of the chromosomes of the 2n complex was duplicated in simply quadruploid cells after chloral treatment. This indicated that in these cells chromosomal duplication by irregularities of distribution, and not by chromosome fragmentation, is the cause of the increase in number. This is an observation of great significance in genetical studies, where interbreeding of 2n and 4n races is often accomplished. M. Navashin ('25) has carried further most skillfully the comparative studies of chromosome forms within a genus (*Crepis*), and has also traced the inheritance of these chromosomes in interspecific crosses, mutations, etc. Indeed, his work probably includes the first application of really critical observations on chromosome form to the analysis of genetical results in relation to the inheritance of specific chromosomes other than those concerned with sex. In this last respect the papers of Blackburn ('24) and of Allen ('19) are the most searching. This list of researches in the field of chromosome form is only partial, and the results embodied in shorter papers by scattered workers have built up a very valuable body of data. My own investigations ('24–'26) began with a study of the changes in form involved in the n and 2n vegetative and the meiotic divisions of *Gasteria*, giving a much more detailed discussion of the various stages than Sakamura had given for *Vicia Faba*. The value of this lay in the indications it gave of the difficulties on analysis of the several stages and in the correlation of the forms assumed by the individual chromosomes in different stages. Later a similar study was reported for *Velthemia*. After finding that there was no recognizable variation among the species of the genus *Gasteria* available, a comparison was offered between the related genera *Gasteria*, *Haworthia*, and *Aloe*, suggesting the probable significant differences. Finally, analyses were prepared of the vegetative complexes of several species in such widely separated genera as *Vicia*, *Galtonia*, *Fritillaria*, *Silphium*, *Allium*, *Sagittaria*, and *Cyrtanthus*, the latter having an especially varied assortment, and there was traced the availability of the criteria used in interpreting the large chromosome types (*Fritillaria*) in genera with small chromosomes (*Eichhornia*, *Coix*, *Aechmea*, and others).

The features which give the chromosomes its characteristic form are first, size; second, the position of attachment of the spindle fiber, which is marked by a constriction (or at least by a paler band) and usually by a flexure; third, constrictions (or bands), unrelated to fiber attachment, which may perhaps intergrade to the fourth class, satellites, which are bodies of varied size attached by elongate fibers to one end of the chromosome. It is too early to state to what degree the fiber-attachment constrictions are general in large chromosomes, but in my studies I have failed to find a case among any of the medium or large chromosome types studied in which a chromosome has a truly terminal fiber attachment or a non-terminal attachment unmarked by a constriction in the second maturation division, in the first pollen grain mitosis, or in somatic mitoses. In those with nearly terminal attachment there is always at least a terminal knob about the base of which attachment seems to be effected. This is

true even of those chromosomes which bear proximal satellites, and in which the attachment constrictions are particularly hard to demonstrate. These and the lesser non-attachment constrictions are rather easily obscured by the shrinkage incident to crude fixations, and they show with the greatest regularity when Flemming-fixed smear preparations are used, or when in sectioned material the cells near the surface are especially considered in adapting the fixing fluid and are treated as the only ones fit for careful analysis. It seems that the fixation of the deeper cells cannot be controlled with a precision sufficient to insure a regular appearance of the critical features of the chromosomes. These features, as well as the satellites, appear independently of the type of fixing fluid used, although some fluids are markedly more dependable than others in the excellence of the results produced.

With respect to the internal structure of the chromosomes I have for some time been studying the maturation and vegetative divisions of *Gasteria*. This



FIGS. 1–11. Drawings of metaphase and anaphase chromosomes showing the tetrad character in *Gasteria*. Magnification about ×2000.

work is still incomplete. A study of *Tradescantia*, *Rhoeo*, and *Podophyllum* begun under my direction by Kaufmann has been partly completed ('25–'26). This exhibits a striking definiteness of internal spiral structure, which Kaufmann traces through various stages. While there is much similarity in these plants with conditions shown in *Gasteria*, the differences, at least at present, appear to suggest quite a range of possibilities in the behavior of what are probably fundamentally the same internal structures. The situation in *Gasteria* is rendered of critical interest by the presence of well defined and absolutely undeniable chromosome tetrads, in the zoological sense of the term. The existence of these

structures in plants has been scouted with some asperity, especially by Gates ('24), but no amount of reiteration of negative evidence can supersede the demonstration offered by *Gasteria*. It has long been known that plant chromosomes often split longitudinally during the first maturation anaphases (Sharp, '26, page 271), and that the appearance of this split may be early or late in the anaphases. The natural conclusion is that this is preceded by a structural differentiation of each homolog preparatory to the separation, but it is not easy to demonstrate this. If this condition is present at or before the metaphase we have essentially that effect which the zoologists term the chromosome tetrad. In *Gasteria* the prophase studies are incomplete, but it appears probable that this tetrad structure will be demonstrable as early as strepsinema. After the nuclear membrane breaks down, recognition of it becomes difficult because of the compact form of the chromosomes; but by the time the plate has been organized it can be seen that actual physical cleavage of the chromosomes is occurring in a plane at right angles to that separating the homologs. This is far advanced for the large chromosomes before they begin to pull apart in anaphase. The smaller chromosomes divide likewise, but more tardily. Figures 3 and 10 represent metaphase conditions. Figures 1, 2, 4–7 illustrate the ways in which the chromosomes draw apart in anaphase; in all cases the separation of the homologs along at least part of their length being preceded by their split into two (right and left) halves. This split is in fact complete in those shown in Figure 7, but in those in Figure 8 the reverse is true. Figure 9 shows a general equatorial view of an entire, early anaphase complex, and in two of the pairs the end view gives a pretty demonstration of the tetrad character. Figure 11 shows some of the smaller chromosome pairs in late anaphase. The process of separation of the homologs is shown in diagrammatic fashion by the models photographed for the accompanying plate (Plate I), explained in the legend. By the completion of anaphase all of the homologs have completely separated into their component halves, as was emphasized in an earlier paper ('24). It has always been difficult to distinguish clearly the separation of homologs from a possible anaphase split, and this difficulty increases as one attempts to study stages close to metaphase. Because of this it is of the utmost importance that the character of the fiber attachment be first established for the individual chromosome type under observation. If this is not done cases of single median attachment will readily pass for incompletely split rods, or more especially split rods will be interpreted as cases of undivided but bent chromosomes, and the essential tetrad character will be overlooked. When it is once understood that the fiber attachment is a normal morphological feature of the chromosome and subject to change only by the usual mutational or evolutionary processes, it becomes easy to use the character of the attachment as a guide in searching for evidence as to the degree to which the metaphase chromosome tetrad has been organized through the plant kingdom. Evidence as to the prophase condition is far harder to secure and will come only with a considerable improvement in the technique applied to these studies.

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Explanation of Plate

PLATE I

MODELS OF THREE TYPES OF FIRST MATURATION DIVISION CHROMOSOMES IN GASTERIA

Fig. 1. Late anaphase, long chromosomes of closely subterminal type, viewed from outside the spindle, with the tetrad split widely open.

Fig. 2. Early anaphase, the same type of chromosome and position, the tetrad split partly opened.

Fig. 3. Metaphase, the same type of chromosome and position, the metaphase tetrad character visible but the elements in contact.

Fig. 4. Late anaphase, the same type of chromosome from the side, showing primarily the separation of the homologs, but the division of each of these recognizable on the right.

Fig. 5. Early anaphase, the same type of chromosome and position, the separation of the homologs evident, the splitting largely obscured because of the position.

Fig. 6. Metaphase, the same type of chromosome and position, the homologs in contact, but almost no trace of the tetrad character visible.

Fig. 7. Late anaphase, the small chromosome type, viewed from outside the spindle, with the tetrad splits nearly completed.

Fig. 8. Early anaphase, the same type of chromosome and position, the tetrad character evident.

Fig. 9. Metaphase, the same type of chromosome and position, the tetrad character visible but the elements in contact.

Fig. 10. Late anaphase, the same type of chromosome nearly from side view, showing primarily the separation of the homologs, but the division of each of these recognizable on the left.

Fig. 11. Early anaphase, the same type of chromosome and position, the separation of the homologs beginning, the splitting of these obscured by the position.

Fig. 12. Metaphase, the same type of chromosome and position, the homologs in contact and the tetrad character invisible.

Fig. 13. Late anaphase, long chromosomes with fiber attachment a considerable distance from the tip, viewed from outside the spindle, with the tetrad splits widely open.

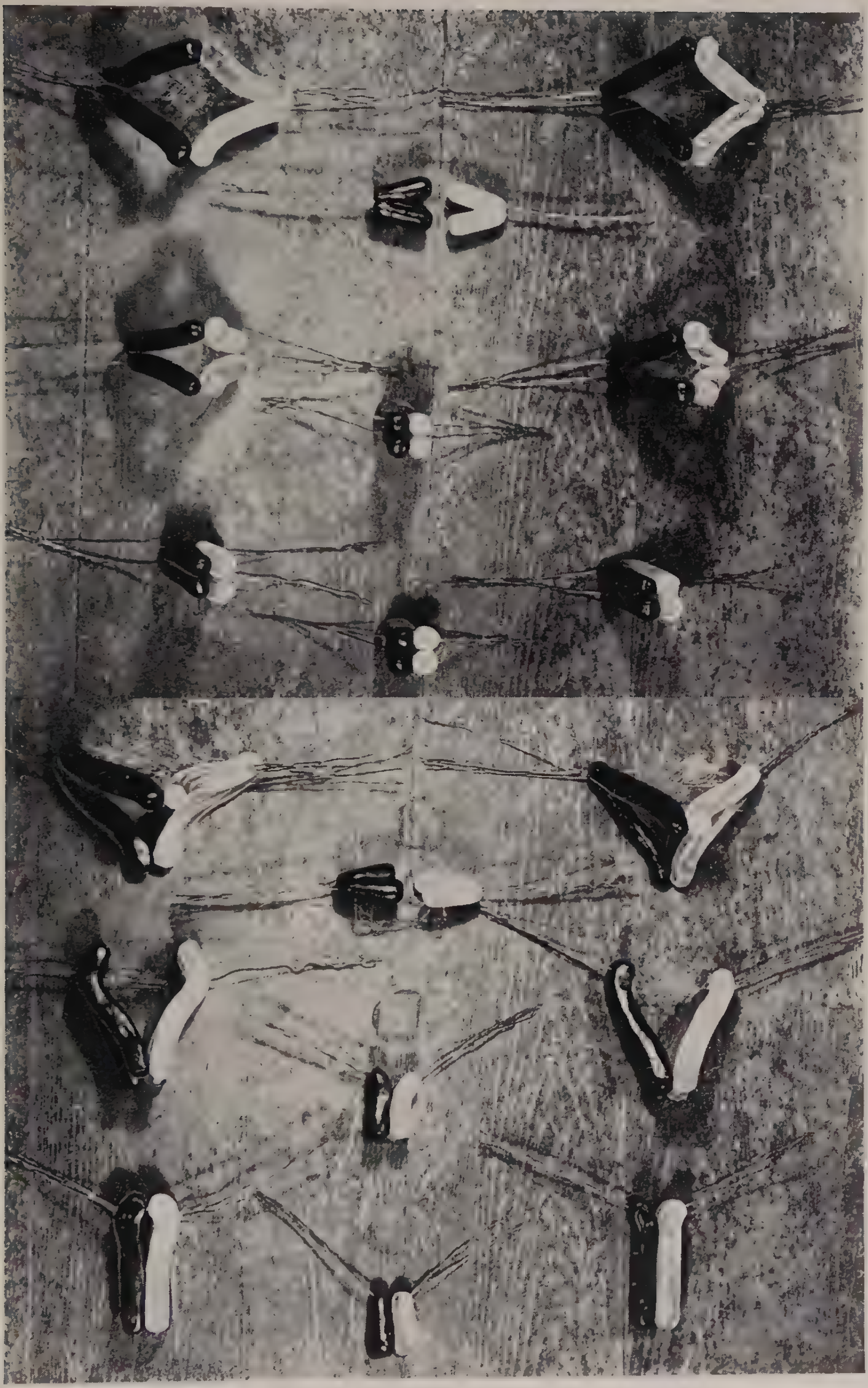
Fig. 14. Early anaphase, the same type of chromosome and position, the tetrad splits partly opened.

Fig. 15. Metaphase, the same type of chromosome and position, the metaphase tetrad character visible but the elements in contact.

Fig. 16. Late anaphase, the same type of chromosome obliquely from the side, showing primarily the separation of the homologs, but the division of each of these recognizable on the right.

Fig. 17. Early anaphase, the same type of chromosome and position, the separation of the homologs evident, the splitting largely obscured because of the position.

Fig. 18. Metaphase, the same type of chromosome from the side, the homologs in contact, but no trace of the tetrad character visible.



TAYLOR: CHROMOSOME STRUCTURE

CYTOLOGICAL STUDIES ON IRRADIATED TISSUES

I. THE INFLUENCE OF RADIUM EMANATION ON THE MICROSPOROGENESIS OF THE LILY[1]

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The increased use of radium emanation in the treatment of malignant diseases has made it desirable to investigate under carefully controlled conditions and with refined cytological technique, the cellular changes that these rays induce. Again, the growing use of filtered radium emanation has been another incentive to undertake this study. It is important to contrast the effects of filtered and non-filtered radium emanation on the cell.

The number of abnormal types of cells found in malignant growths (Levine '25) both in the animal and in the human subject, make those materials impracticable for this purpose. I have selected for this study plant material, such as the sporogenous tissue of the anthers of *Lilium superbum*, *L. auratum*, *L. Harrisii*, and *L. giganteum*. This selection was based on the fact that the life history of this tissue is well known through the studies of Strasburger ('82), Sargant ('97), Mottier ('03), Allen ('05), Guignard ('15), and others. The cells are large and yet the tissue is relatively small. Few atypical cellular structures are found here (West and Lechmere, '15) that would interfere with a correct interpretation of the results. The plant, it appears, presents many advantages over the animal as material for such studies.

The study of the effects of X-rays and radium on plants has been generally confined to seeds and seedlings in their ability to germinate and grow after irradiation. Yet Schober ('96), Lopriore ('98), Seckt ('02), Molisch ('05), and others have studied the influence of these rays on protoplasmic streaming, movement, and other plant responses.

Koernicke ('04, '20), in a series of reports on the effects of radium and X-rays on *Vicia Faba*, *V. sativa*, *Papaver somniferum*, and *Brassica Napus* seeds, claims that retardation of growth is produced some time after the exposure. The time of the appearance of the effects of the irradiation depends upon the intensity of the rays, the subject of the experiment and its physiological condition. Koernicke believes that if the intensity of the irradiation is not too strong, the retardation of growth is temporary. Dry seeds irradiated intensely are stimulated to growth when placed under proper conditions. Seedlings in an advanced

[1] Presented before the International Congress of Plant Sciences, Section of Cytology, Ithaca New York, Aug. 17, 1926.

state of development are not affected. Koernicke's experiments on seeds, although well controlled, lack conviction because of the relatively small number of seeds used. In 1905, the same author made a histological examination of the seedlings of *Vicia Faba* affected by radium, and X-rays, and found that while the shoots of these plants were retarded and the number of leaves reduced, there were no atypical cellular changes to be found. In the roots of these plants there is evidence, he believes, of thickening of the tissue. Roots of *Vicia Faba* and *Pisum sativum* after an irradiation with 5 or 10 mgm. of radium for 1 to 3 days, showed nuclear and cytoplasmic changes. On the second and third days after the irradiation, few divisions were to be observed. Similar observations were made by Grasnick ('18) and by Alberti and Politzer ('24) for irradiated animal tissues.

I have found similar results, reported below, for lily anthers. Koernicke found, in the roots studied, aberrant chromosomes on normal spindles. He also claims that amitoses occur here, likewise multinucleate cells. These results led him to study the effects of radium on cell divisions in the pollen mother cells of *Lilium Martagon.* He used young and old buds which he irradiated for time periods of 1 hour to 3 days, and at short intervals during a period of 1–14 days he prepared the material for cytological study. His controls consisted of anthers of non-irradiated buds of equal size and equal height on the same flower stalk as the irradiated buds. Koernicke reports the presence of clumped chromosomes and of fragmented chromosomes in the sporogenous cells of the irradiated anthers. He describes belated chromosomes in division stages which result in producing the "hour glass" figures. Such effects have been observed in non-irradiated tissue (Levine '16). The spireme stages seemed to be unaffected. The nuclei of vegetative cells appear to be more resistant to irradiation. Cytoplasmic injuries, such as have been described by Zuelzer ('05) and by Packard ('15) for animal cells were not observed.

Fabre ('10) studied the effects of radium on young buds of the lily. He found that intense irradiations inhibited the development of the flower bud and caused it to dry before it attained half its normal size. The ovary and stigma completely atrophied. The anthers, Fabre claims, failed to attain normal size, although the filaments appeared well grown. On examining sections of the anthers, he found that only pollen grains devoid of nuclei were present. The ovary was likewise sterile.

MacDougal, Vail, and Shull ('07) in their study of mutations, variations, and relationships of the Oenotheras, subjected flower buds to various poisonous agencies. Following Gager's ('08) method, they subjected *O. Lamarckiana* flower buds to radium which was fastened to an inflorescence at a distance of 15 to 25 mm. The corollas of many flowers were so affected that they failed to open and fell off prematurely. At a greater distance, the ovary proceeded, but slowly, and normal size was not attained. Seeds formed in such ovaries developed two mutants.

Gager ('08) used radium on seeds of, *Phaseolus, Phleum, Avena, and Lupinus.* He contends that within the limits of an optimum stimulus seeds irradiated show better growth; beyond the optimum, a depression in growth activity

occurs. Gager irradiated the soil, water, and air to determine the effect on the growth of grains. He concluded that if the strength of the radium, the duration of the exposure, and other conditions are suitable, the response shows an excitation of function. With intense irradiation, retardation of growth results.

Wetterer ('12) studied the influence of X-rays on soaked sunflower seeds. He found that seeds exposed to large doses of X-rays failed to germinate, while those receiving a smaller dose produced poor plants. Seeds from these poorer plants showed poor growth. The seeds from the latter were apparently normal and produced plants of normal size.

Congdon ('12) used 8 mgms. of radium bromide to test the effect of the beta rays on the germinating power of dry seeds of a number of plants. The exposure was made for a period of 24 hours at a distance of 1 cm. from the tube. Congdon contends that the beta rays have a greater retarding action than the more rapid electrons.

Molisch ('12) studied the effects of radium and radium emanation on the development of buds of a number of common trees and shrubs. He also studied the effects of the rays on seeds, seedlings, and leaves of mature plants. His observations on buds seem to have convinced him that definite exposure must be made at a definite period in the dormancy of the bud in order to insure a stimulated development. A short exposure early in the resting period of the bud shows no advantage over the controls. An intense irradiation causes injury and even death. Irradiation late in the resting period of the bud has no effect on its development. In the study of the influence of radium and radium emanation on seeds Molisch believes that a light dose acts as a stimulant to development. Leaves and growing points of plants were also influenced by these radio-active substances.

E. Schwarz, ('13) studied the effect of X-rays on plants and animals. He endeavored to determine the optimum irradiation that stimulates growth of seeds of *Vicia Faba* so as to produce the greatest growth in the shortest time. The number of seeds used in his experiments was relatively small. He claims to have observed a stimulative effect on germination resulting from short exposures, while with very short exposures he saw no effect at all. He observed similar results on fertilized eggs of *Ascaris megalocephala*. The eggs when exposed for a few minutes to X-rays formed worms more rapidly than the controls. Schwarz also studied the effects of small doses of X-rays on granulating wounds of twenty human subjects and of rabbits. Using a lead plate to cover part of the wound, the exposed part was treated. According to Schwarz the irradiated portions of the wounds healed more rapidly.

Mottram ('13) studied the action of beta and gamma rays of radium upon the radicles of beans. He found that long exposures stopped the growth of the roots and frequently caused death. The growth for 3 or 4 days following the exposure was due, Mottram believes, not to cell division, but to cell elongation. Exposures of seedlings to radium during the night and day showed that the roots were more vulnerable at night. This he explained on the assumption that cell division occurred more frequently at night.

Miege and Coupé ('14), in studying the effect of X-rays on the development of *Raphanus sativus* and *Lepidium sativum* believe that heavy doses stimulate the development of these plants. The tissues examined show a cuticular thickening. The fibro-vascular bundles were better developed. Komuro ('22, '24) in a series of papers reports the influence of X-rays on the germination of seeds of *Vicia Faba*. The experiments follow the general methods of Koernicke ('04, '05). He exposed the dry and soaked seeds, also the seedlings, to various intensities of Roentgen rays. He reports the stimulative effects of mild doses of irradiation, but finds, as Koernicke did, that more intense raying produces death. Komuro also made cytological studies on the irradiated roots of the seedlings. Asymmetrical divisions, amitoses, and multipolar spindles appeared in the cells of these roots. He compares the atypical cell divisions he found in these rayed roots with those found in cells of malignant tumors.

Sakamura ('20) investigated the nuclear phenomena in the radicle of *Pisum sativum* after exposure to X-rays. Although the division figures observed here were normal, such conditions as the shortening of the chromosomes were observed.

Sierp and Robbers ('22) instituted a series of studies on the effect of X-rays on the growth of the oat plant (*Avena sativa*). They confirm the observation of Koernicke ('04) and others that under the influence of Roentgen rays a slight increase in growth occurs which is followed by retardation. Iven ('25) also obtained similar results on *Vicia Faba equina*. Iven used filtered X-rays and found that the soft beta rays are more intense in their action than the hard gamma rays. Iven also examined his material microscopically and found irregularities in nuclear behavior. Iven claims that plants grown in greenhouses are more sensitive than those grown in the field.

G. Schwarz, Czepa, and Schindler ('24, '25) have re-investigated the question of the effects of X-rays on germination. They used large numbers of seeds of oat, wheat, and bean to determine whether or not the X-rays have a stimulative effect. They followed the methods of earlier workers. These authors contend that light doses of Roentgen rays do not stimulate growth. They point out that the number of seeds used by earlier workers was relatively small and that slight differences in temperature and moisture explain, in a measure, the results obtained. On the basis of a large number of careful measurements, these authors conclude that irradiation with X-rays causes retardation of growth. Light doses have no stimulative effect. They contend that the fluctuating variability of the seed plays an important role in this study. Czepa ('24) emphasizes the observation made in collaboration with Schwarz and Schindler. He X-rayed *Phaseolus* seeds. The stimulative effect of X-rays on growth of seeds finds no support in his results.

Emmy Stein ('22) studied the effect of a filtered radium compound on the development of a young Sippe of *Antirrhinum*. Plants irradiated for more than $2\frac{1}{2}$ hours displayed a strong but otherwise normal growth after a temporary retardation. The flower first formed lacked pollen. Later flowers were normal and capable of forming fruit. Irradiation for over 5 hours caused complete inhibition

of the growth of the vegetative point. In these plants there was no further growth except a stretching of the main shoot. Irradiation of 85 hours retarded the growth of the plant so as to form a rosette. Miss Stein also studied the effects of radium on seeds which she exposed for short intervals. She describes pathological changes in the cotyledons and changes in the color of the flower and in shape and size of leaf and stem.

Stephanie Herzfeld ('23) studied the effect of Roentgen rays on *Leptobryum pyriforme*. She arranged the mosses in open Petri dishes and exposed them all at the same time for different periods. The plants were prepared for microscopical examination at definite periods after the irradiation. Visible effects were noted in the form of new leaf formations two days after the irradiation. Those receiving longer exposures produced modified leaves in the form of trichomes and globular bodies. Sections of the apical cell convince Miss Herzfeld that the irradiation affected it, causing a shortening of the plant axis and an increase of the leaves and trichomes. She believes that the X-rays induced the development of new characteristics in these plants which resemble those of the associated or related species found on high mountains.

Weber ('22) studied the influence of X-rays on twigs of *Syringa* in order to reduce, if possible, the resting period of the winter buds. He observed that large doses of X-rays had the desired effect, yet after a period of 3 weeks the base of the bud necrotized.

Maud Williams ('23) prepared strips from the upper surface of petioles of *Saxifraga umbrosa* which she exposed to X-rays. She found that small doses of X-rays caused depression of the rate of circulation of the fluids within the cells.

The study of the effect of X-rays and radium emanation on pathological conditions in plants such as crown gall and club root, was attempted by Levin and Levine ('17, '18). Stems of *Ricinus communis* inoculated with the crown gall organism *Bacterium tumefaciens* produced in all cases uniformly large intumescences. Such growths irradiated by X-rays in doses used in the treatment of malignant cancer of the human subject were retarded in growth and were smaller in comparison with the controls.

In two other reports Levin and Levine ('22, '24) describe the effect of burying glass capillaries containing small quantities of radium emanation in the hyperplasias, crown gall, and club root, as well as in the normal growing shoots of the tobacco and root of the turnip. In all cases small areas of necrotic tissue resulted about the "seeds" so implanted. The hyperplastic tissue in the immediate vicinity of the capillary failed to undergo further proliferation. Levine ('25) reports further the observations on the effect of radium emanation implantations in young and old crown galls on the geranium. Gross and microscopical studies were made of galls irradiated at frequent intervals, from 1 hour to 30 days. Cells in the immediate vicinity of the "seeds" are destroyed while cells further removed from the source of the rays are apparently normal. No aberrant or atypical cells were found in these irradiated galls.

Experimental data on the effects of X-rays and radium have been contrib-

uted by a large number of zoologists and human pathologists. It is beyond the scope of this brief review to give the results here. Only such mention is made of this work as will effect a better understanding of the problem under consideration.

Perthes ('04) on the basis of his observations of the effects of X-rays on wounds, finds, unlike Schwarz, E. ('13), that the treatment delays healing. To study the effects of X-rays, he employed a large number of fertilized eggs of *Ascaris megalocephala*. He found that the intensely irradiated eggs produced masses of undifferentiated cells or imperfectly formed worms. With lighter doses the injuries induced by the X-rays were not uniform. Some worms developed normally while other eggs developed into irregular masses of cells. The strongest irradiation he used was non lethal. Perthes, like Koernicke ('04, '05), also contends that the effects of the rays appear after a latent period. Microscopical examination showed fragmentation of chromosomes. Similar results were obtained with radium.

Lazarus-Barlow and Bonney ('09) repeated Perthes' experiments. They used as their sources of radio activity, X-rays, radium, thorium, and uranium. They contend that short exposures to X-rays of fertilized eggs of *A. megalocephala* induce a more rapid rate of division, while long exposures retard development. Evidences of retardation, they claimed, were obtained with thorium and uranium. Lazarus-Barlow and Becton ('13) confirm the earlier results obtained in their laboratory.

Stevens ('09) studied the effect of ultra-violet light on the development of fertilized eggs of *A. megalocephala* and found that an exposure too short to prevent cleavage results in fragmentation of the chromosomes, delay in cleavage, and absence of germ cells in some embryos. Chromosomes in mitosis remain unchanged after a short exposure to ultra-violet light or in case of a long exposure when fixed before disintegration of the cytoplasm sets in.

Paula Hertwig ('11) reports a number of different abnormal karyokinetic figures which appear in the radiumized eggs of this much investigated worm. Payne ('13) exposed *A. megalocephala univalens* to radium rays. Payne also confirms Perthes ('04), P. Hertwig ('11), and others, that the chromosomes fragment under the influence of radium. Payne states that the multinucleate cells found in the dividing radiumized eggs may not be due to radium effects alone.

Margarite Zuelzer ('05) studied the effects of a radium compound on the structures of a number of protozoa. Short exposures of *Pelomyxa palustris* to the rays of this compound increased its cytoplasmic streaming; but later this ceased. The organism rounded up, filled with water, and burst. *Spirostomum ambiguum* appeared unaffected after a 24-hour exposure. After 36 hours the macronucleus changes so that its usual rosette form becomes clumped into 4–7 deeply staining bodies. The cytoplasm becomes vacuolate and filled with water. Miss Zuelzer contends that radium first has a stimulative effect. This is followed by inactivity and destruction. Some protozoa are capable of recovering from the effects of irradiation. Long exposure destroys them.

Packard ('15) studied the effects of beta and gamma rays of radium on the eggs of *Nereis limbata*, *Arbacia punctulata*, *Drosophila ampelophila*, and *Paramoecium caudatum*. The gamma ray activity undoubtedly associated with secondary rays produced slight acceleration in the rate of cell division, in the sea urchin, while the eggs of *Nereis*, fruit fly, and protozoa are unaffected. The action of the beta rays, however, induced peripheral changes in the protoplasm followed by death. These results appear to be in accord with the observation of Miss Zuelzer ('05) for certain protozoa. Abnormalities in development occur after prolonged irradiation. The type of abnormality produced by radium is frequently found in over-crowded control cultures.

Packard ('18) observed the effects of 50 mgms. of radium bromide on the development of *Chaetopterus* eggs. He claims that if the egg is radiated before insemenation, its nucleus is destroyed so that it plays no part in development after fertilization. He followed carefully the disintegration of the egg nucleus and showed that the sperm nucleus takes possession of the egg cytoplasm. The development is androgenetic. Packard's studies supplement the observations of G. Hertwig ('11), on the development of irradiated eggs and sperm cells of the frog, and P. Hertwig's ('16) findings on the Triton. Mavor and DeForest ('24) also studied the relative susceptibility of eggs of *Arbacia punctulata* to X-rays. They found that the smallest doses caused retardation, that the amount of retardation increases with the dose, and that for the same exposure the greater retardation results when the sperm cells are irradiated than when the eggs are exposed.

Grasnick ('18) studied the effects of radium and mesothorium on all the tissues of the tails of *Rana fusca* and *Amblystoma tigrinum* 17½ hours to 15 days after irradiation. He observed a disturbance of the circulation in some of the capillaries followed by atrophic changes in the tail several days after the treatment. He irradiated the tails of his animals for 1½ hours with silver filtered mesothorium. These showed no effects after 15 days. Specimens fixed a short time after irradiation showed the absence of dividing cells. There were small, homogeneously stained pycnotic cells. Fifteen days later a small number of division stages were found. In animals prepared for microscopical examination a long period after irradiation, cytoplasmic changes were observed in the cells although the mitotic figures appeared normal. Grasnick contends that, in general, only mitotic stages or nuclei approaching division are affected by raying. The resting nuclei resist the influences of the rays. The subsequent appearance of normal cell divisions after the irradiations indicates this resisting power of the resting cell. The author figures nuclei of resting cells in which the chromatin is massed toward the nuclear membrane with a fine band of chromatin running around it forming the "signet ring" effect. He contends that these nuclei were about to divide. Grasnick's observation on the effects of rays on the cytoplasm are in accord with those of Zuelzer ('05) and Packard ('18).

Alverdes ('21) exposed eggs and sperm of *Cyclops viridis* to radium bromide for intervals of 10 minutes to 13 days. He was able to induce cytological changes

in the embryos when the male cells were exposed, while no change occured when the eggs were rayed. He also described aberrant types of chromosomes.

Alberti and Politzer ('24,) studied the effects of slightly filtered X-rays on the corneal epithelium of Urodel larvae. The exposures were 10–40 minutes and fixations were made at 2–3 hour intervals for 10 hours, and then daily fixations were made for 5 days. Alberti and Politzer believe that three stages are to be recognized in irradiated tissues. The first stage which they call the primary effect follows 2 hours after the irradiation, and is characterized by the presence of pycnotic cells, pseudo-amitosis and other aberrant nuclear phenomena. This is followed by a period of inactivity during which time no mitoses appear. The duration of this period is not definite. The third stage referred to by these authors shows the secondary effects which are characterized by disturbances in the movements of chromosomes to the poles. Delayed chromosomes, such as those observed by Koernicke ('05) in *Lilium Martagon*, resulting in small nuclei and "hour glass" effects, are also noted by these authors in the treated larvae. The spireme stages and asters are usually normal in appearance. The results of these two authors are in harmony with the observations of Grasnick ('18).

Sittenfield and Balbina Johnson ('25) studied the effect of radium emanation of 0.1–5 millicuries at distances of 1–2 mm. on cultures of cells of the heart and spleen of chicks 7–20 days old, and tissue of Flexner-Jobling rat carcinoma. Of the 100 radiumized cultures, 49 per cent showed no growth; in 30 per cent growth occured in the cells nearest the "seed"; while in 21 per cent the growth was fair to normal. These authors contend that their cultures were affected by very small quantities of radium emanation in the same proportion and manner as with larger amounts.

Strangeways ('25) also studied the effect of X-rays on cells *in vitro*. He also used the unopened egg of the hen. He states that doses of rays have an inhibiting effect on cell division and prevent new cells from coming into mitosis. Strangeways contends that there is no definite evidence that cells in actual mitosis are more vulnerable than other stages.

An analysis of the above cited literature from the plant and animal fields of investigation shows in all cases that the chromatin material in division stages is most susceptible to radio-active substance. The most frequently found effects are fragmentation and clumping of chromosomes, Perthes, ('04), Zuelzer ('05), P. Hertwig ('11), belated chromosomes, Koernicke ('04, '05), Grasnick ('18), Alberti and Politzer ('24, '25). Multipolar spindles, multinucleate cells, and pseudo amitosis were observed by a number of investigators. These conditions are found in pathological tissue and may be induced according to Galeotti ('93) and Schottländer ('88) by various agencies.

The cytoplasm in most cases of irradiated tissue has been found to be unaffected. However, Zuelzer ('05), Packard ('15), Grasnick ('18), and others show that cytoplasmic disintegration may occur.

With respect to the effect of X-rays, radium, and other radio-active substances on the growth of seeds and seedlings, there is no unanimity of opinion.

The contention is held by some that small doses have a stimulative effect while larger doses retard growth. All agree that large doses retard growth and cause death. The same is true for the results of the study of the effects of the radioactive substances on the eggs of invertebrates.

In mature plants irradiation causes mutations (MacDougal '07) and aberrant forms (Stein '22, Herzfeld '23).

Materials and Methods

I am reporting here the materials and methods used in the study of the effects of radium emanation on the development of the sporogenous tissue in the anthers of the lily. Radium emanation is the product of the decomposition of radium. It is an elementary body in the state of a heavy gas. It in turn decomposes and liberates alpha, beta, and gamma rays. The radium emanation is collected from the decomposing radium by means of appropriate apparatus in small glass capillaries commonly referred to as "seeds." These tubules measure 3–5 mms. in length and 0.25 mm. or less in diameter. The alpha rays do not penetrate the walls of the glass capillaries. The beta and gamma rays pass through the glass.

Within the last few years, radium emanation has been collected in gold capillaries, a trifle larger in size than the glass ones. The purpose of the gold is to serve as a filter so that only the hard or gamma rays will act upon the tissue to be irradiated. However, the effects of beta rays are not entirely eliminated by this method.

In the treatment of malignant diseases, the glass or gold capillaries containing the radium emanation are implanted in the growth by means of a trocar. In the experiments reported below, glass capillaries were used. Small hollow cylinders of platinum, silver, and aluminum were used as filters. The walls of these tubes are 0.5 mm. in thickness. The lumen of the filter is just large enough to permit the introduction of the glass capillary containing the radium emanation. The glass capillaries are kept in place by sealing the openings of the filters with small plugs of lead, kept in place by paraffin. Empty tubes of aluminum were used as controls.

It is calculated that the filtering power of gold, platinum, and silver 0.5 mm. in thickness is approximately the same for the beta rays. The aluminum permits about 50 per cent of the beta rays to pass through.

The plants used in this work are the common species of Easter Lily (*Lilium Harrisii* and *L. giganteum*). These were grown in the greenhouse from 1923 to the present. *L. auratum* bulbs were grown in the garden connected with my laboratory. Bulbs of *L. superbum* were collected in the field and also grown in the garden for a number of years. The plants grew normally and produced an abundance of flowers in season. Over one hundred plants were kept under observation.

The common method of implanting the "seeds" by means of a trocar was not used directly on the anthers, because of the injury induced and the small size of the anthers. However, bare "seeds" were implanted in the ovary. The tip of the flower bud was removed above the stigma. A fine sterile needle was

introduced through the center of the ovary parallel with the long axis of the bud. With the aid of sterile forceps and needle, the "seed" was placed in the opening made. By this method all the anthers were approximately the same distance from the source of the irradiation. In small buds this distance was 1–2 mms. Buds of approximately the same size and the same height on the plant served as controls. This type of control was used by Koernicke ('05). These controls were satisfactory when the exposures were made for short periods and the fixations followed shortly afterward. For long exposures, this method was not entirely satisfactory, for while the buds of the same size contain anthers in approximately the same stage of development, the variation in the development of the sporogenous tissue in the same anther frequently interferes with the best interpretation of the results.

I used a more direct method of control and exposure. Having determined approximately the stage of development of the sporogenous tissue of the anther, I made three incisions in the bud,—a short transverse opening at the base and two vertical incisions parallel with the sides of the bud, thus forming a triangular shaped flap attached at the apex of the bud. The flap opened at the base, exposing two anthers. These were carefully removed by severing the filaments. The ovary was also removed. This formed a cavity about 2–5 mms., or more in diameter, depending upon the size of the bud. The removed anthers served as controls. They were prepared for microscopical examination, as described below. The "seed" was then carefully introduced into the bud without injury to the remaining anthers. The "seed" was generally placed next to the anther or between two adjacent anthers. A filtered "seed" was so placed as to touch gently all the anthers. The flap of the perianth was put in place. A label indicating the content of the "seed," and the hour of implantation was tied carefully about the bud.

Fifty-two experiments with buds, measuring from 5–20 mms., in all stages of development from the premeiotic division stages to mature pollen grains, were exposed to bare, and silver, platinum, and aluminum covered "seeds" containing 0.25–2 millicurie of radium emanation. The duration of the exposures lasted 1 hour to 10 days, and fixations at intervals of 12, 16, 19, 24, 48 hours, 3, 5, 7, and 10 days were made.

In 32 experiments, I exposed anthers of all sizes to filtered and unfiltered "seeds" for 1, 2, 3, 4, 12, 19, 24, 33, 48 hours, and fixed the materials 24–72 hours later, at intervals of 12 hours.

I have also studied a large number of buds in these 2 series with empty tubules to serve as a check against the effects of the operation and the possible effects of the metal. I have been unable to study the effects of different quantities of radium emanation, but in all cases I tried to use larger quantities for short exposures and small quantities for long exposures.

The exposed and control anthers were generally removed from the bud before fixing. I also fixed the entire bud for purposes of orientation. Flemming's stronger solution and Carnoy's solution were used almost exclusively, as the fixing agents. The material was imbedded in paraffin and sections 5–20μ were

made. All preparations fixed in osmic acid were bleached in sections in a solution of 4 parts of 80 per cent alcohol to 1 part of hydrogen peroxide. Heidenhain's iron alum haematoxylin and Kultschitzky's haematoxylin stains were used. Methylene blue counter stained with safranin in oil of cloves proved useful in young stages. The best preparations were obtained with Flemming's triple stain after Flemming's fixation.

Observations

It is conceded by all students interested in the problem of the biological influence of X-rays and radium on cell structures, that long exposures to doses of X-rays and radium induces necrosis of the cells and frequently death of the entire organism. The question of the effects of filtered and unfiltered "seeds" of radium emanation on cell structures is still unsettled. Levin and Levine ('22, '24), Levine ('25), Ewing ('26), and others, have shown that implantations of bare "seeds" produces necrosis of the tissue in the immediate vicinity of the "seeds." In tissues of higher animals, the results are the same as in plants, but they are modified, it appears, by wandering cells, the blood, and lymph streams.

I am reporting here studies on the effects of bare and filtered "seeds" of radium emanation on the changes in the cellular structures of the sporogenous tissue of the anthers of *Lilium Harrisii*, *L. giganteum*, *L. superbum*, and *L. auratum*. I have emphasized in my present studies the effects of the radium emanation on the young sporogenous cells in the anthers, because of the great variety of stages in cell division one may find at the same time. The effects of the emanation on the principal stages in the reduction divisions have been studied also. The quantity of radium emanation per "seed" I used is small, but equals that employed by the radio therapeutist. The general practice, however, is to implant a number of these "seeds," at one time, at convenient distances apart, depending upon the size of the growth to be affected. The method used here differs also from that used by the radio therapeutist, in that the glass capillaries of radium emanation were not buried in the anthers, but were placed in close proximity to them.

PREHETEROTYPIC DIVISIONS

The normal development of the sporogenous tissue of the anther of the lily from the resting pollen mother-cells to the mature pollen grains is well known. I have studied very young buds of *L. Harrisii*, *L. superbum*, *L. giganteum*, and *L. auratum*. In these buds the anthers measured 3–5 mms. in length. The distance between the two adjacent anthers is relatively negligible, while the distance between the two most separated is approximately 1.5–3.5 mms. Serial sections of these anthers already show two well differentiated layers of tissue. The inner layer is undoubtedly the anlage of the sporogenous tissue, while the outer layer forms the walls of the anther sac. The inner layer of cells is characterized by large nuclei, richer in chromatin material, and a fine granular cytoplasm. These cells stain rapidly and heavily. These young sporogenous cells grow rapidly, and many division stages are to be found. The division figures are clear, well formed, and as many as five to seven dividing cells may be observed

in a single field under the low power of the microscope. The vegetative cells of these young anthers, and parts of the floral envelope, frequently fixed for purposes of orientation, show beautiful, clear, karyokinetic figures. No abnormalities in cell division are found in these anthers at this stage.

In such young buds, bare "seeds" containing 0.25–3.3 millicurie, were introduced through the center of the ovary, or more frequently the bud was carefully opened as described above; two anthers and the ovary were removed, and the "seed" was substituted for them. The exposures were made for periods of 1 hour to 3 days. Fixations were made at intervals of 1 hour for the first 4 hours and then at intervals of 6 hours for 3 days. The fixation was made immediately after the completion of the exposure. The 2 anthers first removed served as controls. They were carefully fixed and sectioned. They served as a good index of the condition of the remaining anthers in the bud at the time the exposure began.

The irradiated anthers exposed for 1 hour showed changes in the structures of the cells. The chromosomes in division were apparently most affected. These bodies, which are long, rod-shaped structures in normal anthers, became clumped and fused together after an hour of irradiation from a bare "seed." They stain diffusely, and very frequently the view of the spindle fibers becomes obliterated by them. The prophase stages and the spiremes are not visibly affected. Figure 1 represents a section of an anther of *L. superbum* exposed as described above. In this bud 3.3 millicurie of radium emanation was introduced, for 1 hour. Similar results were obtained with smaller quantities of the emanation for the same length of exposure. In the control anthers, a section of which is shown in Figure 2, normal division stages appear.

Anthers of this size, exposed for 2, 3, 4, 12, and 19 hours, show the same results. When young anthers are examined after an exposure of over 24 hours to bare "seeds" containing 0.2–1.5 millicuries of radium emanation, no dividing cells are to be found in the young sporogenous tissue. The vegetative basal portion of the bud and anther also show a noticeable decrease in the number of dividing cells. I have searched diligently serial sections of these tissues. Evidences of only a very few disintegrating cells are to be found. It must be noted that in normal young anther sacs, at the stage in question, one frequently finds cells in which the structures are poorly differentiated. These cells are generally smaller and show evidence of a tendency to disintegrate. That more of these cells appear in irradiated tissue described above, was difficult to determine. The control anthers fixed 24 hours earlier showed a number of cells in all stages of division (Fig. 2). Buds of a size equal to, and fixed simultaneously with the irradiated one, show active division stages in the sporogenous tissue, as well as in other somatic cells.

These observations are in accord with those of Grasnick ('18), and Alberti and Politzer ('24), in their studies with X-rays and radium on animal cells. These authors found that after irradiation of animal cells, there appeared a period in which no division of cells occurred. In my preparation I found no

pycnotic cells at this stage, although the poorly differentiated cells mentioned above are present.

In these young anthers, I have also noted changes in the resting nuclei of cells of the sporogenous tissue, after an exposure of 24 hours or more. The chromatin material in these cells showed evidence of clumping. The chromatin in these irradiated cells becomes peripherally distributed in the nucleus. The nucleus under binocular observation appears to be vacuolate. It seems that the vacuoles increase in size and force the chromatin material toward the nuclear membrane. I am not confusing this stage with the early prophases of the pollen mother cells in which the peripheral distribution of the nuclear reticulum has been described (Allen '05). In carefully differentiated preparations, the normal peripheral distribution of the chromatin material in the nucleus may be distinguished from the pathological condition apparently induced here by the irradiation.

Figure 3 represents a section through an anther of *L. auratum*, 0.6 mm. long, exposed to 0.49 millicurie of radium emanation in a bare glass capillary for 24 hours. No spireme stages are found, although rod-shaped fragments of chromatin substance may be seen. The chromatin material is clumped and distributed along the nuclear membrane. Similar results were obtained in many other anthers. In Figure 4, another view, under high powers of magnification, is shown, of an anther of *L. superbum* 0.5 cm. long, and exposed to 0.7 millicurie of radium emanation, for 24 hours. Quite frequently the entire chromatin mass may be found to be contracted, and appears to lie to one side of the nucleus.

I have studied with care the cytoplasm of these young cells. At this stage the cytoplasm is made up of a fine granular reticulum which stains in my preparations with Flemming's triple stain, a faint orange color. Vacuoles are relatively few and small. The irradiated cells up to 30 hours of exposure show no variation from the normal. Occasionally one finds fibrillar strands with coarser granules. These conditions are found in cells of the controls. In young anthers exposed for over 30 hours sporogenous cells appear with highly vacuolate cytoplasm (Fig. 7). The vacuoles are circular in outline, but frequently become confluent. The nuclei in these cells frequently show the chromatin material clumped as a homogenous, safranin-staining body. These preparations are not abundant. No apparent mechanical injury to the anthers was noticed in the gross specimens at the time of fixation.

Preparations of anthers exposed for 48 hours and 72 hours show no other changes than those which have been observed in the material described above. A comparative study of the control anthers and the ones irradiated for a period of 3 days show undeniably that the effect has been one of injury and retardation rather than stimulation. The ultimate fate of such preparations varies. A number of the young buds dried and fell off. I have sectioned some of these buds exposed for 7 and 10 days, and found vestiges of anther tissue still viable. The sporogenous tissue was entirely gone. It appears that the vacuolization of the cytoplasm may be an early stage in the death of the cell, as shown by Zuelzer ('05) and Packard ('18) for the irradiated animal cell.

A striking result is the rapid effect obtained with the unfiltered radium emanation on dividing nuclei. It must be borne in mind that my observations were made on tissues directly in contact with the radium emanation container. The size of the young anthers made it possible to influence the entire structure. In older buds the part of the anther directly exposed was especially studied. Evidence of the failure of cell division after 24 hours of irradiation is also found here. After 24 hours of irradiation the sporogenous cells show chromatin and cytoplasmic changes. No spireme stages are to be found. I have used varying small quantities of radium emanation, but I have been unable to recognize differences in the reaction. Irradiated crown gall tissue shows larger necrotic areas for given periods of time, with large quantities of the radio-active substance (Levine '25).

The development of young sporogenous tissue in the anthers of the species of lilies under investigation was studied under the influence of small quantities of radium emanation filtered with 0.5 mm. thickness of aluminum, platinum, and silver. The aluminum filtrations of radium emanation gave results which were indistinguishable from those obtained with the bare glass capillaries. The radium emanation filtered through the platinum and silver screens gave practically similar results. The action of the filtered radium emanation was not as great as that from the bare "seeds."

Platinum and silver filtered "seeds" containing 0.2–2 millicuries of radium emanation were introduced into young buds after 2 anthers and the ovary were removed. These were used as controls. The metal-covered glass tubule containing the radium emanation was placed in close contact with the remaining 4 anthers. Exposures lasting from 1 hour to 16 hours were made, after which the anthers were removed and prepared for microscopical examination. A study of the anthers revealed no appreciable difference when compared with the controls. The division stages were normal and there appeared to be as many dividing cells in the exposed anthers as in the controls. In another series of studies on these young anthers, with filtered irradiation, the exposures were made in the same way, with the exception that the anthers were fixed 24 hours to 72 hours later. In these anthers few dividing cells are found. The nuclei present the same appearance as I have described for the cells exposed to the non-filtered irradiations. There is a general clumping of the chromatin, and its peripheral distribution in the nucleus is quite evident. The controls show many dividing nuclei.

Figure 6 represents a portion of a young anther of *L. auratum* which was exposed to 1.7 millicuries of radium emanation, filtered through platinum for 1 hour. The anthers were removed 24 hours later for examination. The peripheral distribution of the heavily stained chromatin is the outstanding feature in this preparation. No division figures are to be noted, though occasionally a diffusedly stained spireme stage is to be seen.

In Figure 7 a portion of anther of *L. superbum* is shown. It was exposed to 1.1 millicurie of radium filtered through platinum 0.5 mm. in thickness. The exposure lasted $19\frac{1}{4}$ hours, and the material was fixed 73 hours later. In this preparation there is noticeable a difference in sizes of the sporogenous cells.

The smaller cells seem to show the effect of being crowded by the larger cells. The nuclei stain heavily in some cells while in others large vacuoles fill the centers. A large number of these irradiated buds showed marked retardation when contrasted with the buds on the same plants. Buds from which 2 anthers were removed, and an empty tube of aluminum was inserted, were also studied under the same conditions described above for the irradiated buds. These controls showed very slight ratardation in growth. The remaining anthers were normal, and the cells were actively dividing.

From a study of sections of many young buds and anthers, irradiated with and without filters, it appears that the action of unfiltered radium emanation is more rapid and more intense than the filtered radium emanation, also, that the quantities of radium emanation used here were too large to bring out, after long exposures, the difference between the two types of irradiation. In the vegetative tissue of the buds studied, it appears to me that much more necrosis occurred about the stub of the removed ovary and anthers after introducing a bare "seed," than after introducing a filtered one for the same time. This observation is in accord with the report of Ewing ('26), in whose laboratory larger quantities of gold and platinum filtered radium emanation have been used on animal and human tissue. The question is still open as to which is the more desirable agent for use in the treatment of malignant diseases (Levin '24).

POLLEN MOTHER CELLS

The young pollen mother cells in the early stages of development are frequently found associated with cells which are undergoing the last preheterotypic division. In anthers of *L. superbum* 0.7–1 cm. in length, such conditions are observed in my cultures. Irradiation of such anthers with unfiltered radiations of 0.5–3.3 millicurie for periods of 1 hour to 3 days, examined at intervals of 12 hours, showed no visible effect on the nuclei of the pollen mother cells in the prophase stages. The dividing nuclei are affected in the same way as the nuclei of the younger cells. The chromosomes are clumped and fused. They stain diffusely. Figure 8 represents a section through an anther exposed to 3.3 millicuries of radium emanation for 1 hour. The chromosomes in division appear in 2 cells in 2 different stages. The cell in the center of the figure shows the chromosomes in the metaphase stage. They are clumped, forming an irregular mass. Only part of the cell is shown in this section. The division figure seen in the lower cell is apparently in the anaphase stage. The individual chromosomes are beyond recognition. They are fused, swollen, and have taken the stain very heavily.

In these larger and older anthers, in the 4 species of lilies studied, I have seen in my control specimens division stages in which chromosomes are left on the spindle. This condition has been described by a number of authors in anthers of a number of apparently normal plants (Levine '16). These belated chromosomes are described by Koernicke ('05), in the reduction divisions of *Lilium Martagon* as the result of irradiation. They have also been described in the irradiated animal cells. I have followed these belated chromosomes in anthers in

control buds subjected to empty tubes for a period of 5 days. When the chromosome mass is left in the central portion of the spindle, it appears that a continuous membrane is formed about the chromosomes at the poles enveloping the chromosome body on the spindle, forming the "hour glass" figure. When the chromosome mass is left on the peripheral fibers of the spindle, an independent nuclear membrane is formed about it. This gives rise to a dwarf nucleus (Fig.9). Frequently one or more chromosome masses are left on the periphery of the spindle, in which case a number of dwarf nuclei are formed. I have seen in these preparations as many as 4 dwarf nuclei in a cell. Cytokinesis is apparently normal. I have been unable to find evidence of this phenomenon in the irradiated anthers at this stage. It is possible that these dwarf nuclei and their precursory stages are obscured under the influence of the irradiation.

At the time the microsporocyte reaches maturity, they separate more or less, and show a tendency to become ovoid. The chromatin material has formed a delicate network. The cytoplasm has a granular consistency with occasional fibril strands. These cells, I find, are very resistant to the effects of the irradiation. The radium emanation in bare capillaries was applied directly to the anther to be studied. The distance between 2 adjacent anthers is small, less than 1 mm., while the distance between two farthest apart is 3–5 mms. All anthers in such buds are not equally affected. Figure 10 represents a portion of an anther sac showing well differentiated nuclei in the pollen mother cells. The chromatin network is evenly distributed through the nuclear cavity, and stains a delicate gentian violet. The irregularly shaped nucleoli, of which there are as many as 3, stain a ruby red. The cytoplasm is delicately stained and presents a fine reticulate, granular appearance.

The anther in close proximity to the "seed" containing 0.4 millicurie of radium emanation for 3 days frequently shows pollen mother cells which are unquestionably affected by the irradiation. Although prepared in the same fixing agent, they show considerable plasmolysis and give the impression of marked cytoplasmic contraction. The cytoplasm in these cells stains heavily a reddish color. The cytoplasmic reticulum can be made out with difficulty (Fig. 11). The chromatin material has gathered in the center of the nucleus, apparently about a nucleolus, with coarse and fine portions of the nuclear linin radiating from it. Frequently, the entire irregularly shaped chromatin mass is found on the periphery of the nucleus. The cells slightly out of the zone of the irradiation in the same anther show aberrant chromosome clumping in division stages, which I shall describe below. The prophase stages of the pollen mother cell are apparently resistant to the irradiation. I have been unable to observe these aberrant stages in living material.

The effect of radium emanation filtered through platinum and silver was tested out on these resting pollen mother cells. The exposures were made with 0.2–2 millicuries of radium emanation for 2–24 hours at intervals of 6 hours. The fixations were made 24 hours, 48 hours, and 72 hours after the exposure. In no case were there any marked effects to be observed in the chromatin, cytoplasm, or any other structure that could be attributed to the influence of the

irradiation. In some anthers there was slight evidence of a greater accumulation of chromatin toward the periphery of the nucleus. Controls showed similar effects. I was unable to note transitional stages which would indicate any other step in the development of these irradiated cells, other than a slight increase in size.

Preparations exposed for 7 days to 1.1 millicuries of radium emanation filtered through 0.5 mm. thickness of silver were also observed. A large number of the microsporocytes were destroyed. The remaining cells farthest away from the source of the irradiation were injured. The cell walls were angular, although some had rounded out. The cell walls were thickened, but not sculptured. The nuclei appear to be smaller, and frequently the nuclear membranes appear irregular in shape.

In some cells the chromatin material is coagulated and appears as a reddish homogeneously stained body. In other cases, such as shown in Figure 12, the nuclear material forms a distinct, yet fragmented, network consisting of large and small masses of chromatin. The cytoplasm is markedly vacuolate. How the destruction of some cells in an anther at this stage affects the development of other cells is not altogether clear, but that interference occurs here appears evident. In the higher animals and man, the occlusion of a blood vessel by radio-active substances undoubtedly affects the surrounding cells. It appears that radium emanation in short exposures has little effect on the early prophases in the microsporocyte, while relatively large doses of the radio-active substance, acting for long periods, produce necrosis. It appears from the study of cells at this stage that growth has been inhibited. A large number of these irradiated anthers fail to develop. Anther sacs have been found in which the sporogenous tissue has completely disintegrated, and the cell debris only is to be found in the sac.

An examination of the control anthers showed that I had been able to subject pollen mother cells, during the leptonema, synizesis, and pachynema stages to radium emanation. Frequently cells in these stages are found in the same anther sac. Another locule in the same anther may show only one or the other stage. Figure 13 represents a portion of a section through a control anther of *L. Harrisii*. The pollen mother cells are in the leptotene stage. The dual nature of the chromatin band may be seen in some cells. In other cells in this anther the chromatin reticulum is still seen in the form of thickened knots.

Anthers directly in contact with bare glass capillaries containing 0.4 to 0.8 millicurie of radium emanation for 4, 12, 18, and 24 hours were studied. The anthers in the irradiated bud, but farther removed from the "seed," were also studied. They failed to show any effect. The sporogenous cells in the anthers which were nearest the "seed" showed the greatest effects, although the results were not uniform in this stage. Marked changes were noted in some anthers of *L. Harrisii*, irradiated for 18 hours with 0.8 millicurie of radium emanation. In these buds some of the pollen mother-cells show marked aberrant changes in nuclear structure. The spireme band has been changed into an irregularly shaped coagulated homogeneous mass, as shown in Figure 14. It now occupies a

peripheral position in the nucleus and resembles a stage in synizesis. Occasionally a globular mass resembling a nucleole may be seen lying outside of the irregular mass of chromatin. That these are not normal, or unaffected stages, is seen by the shape of the chromatin mass, and frequently, by the changes seen in the cytoplasm. There appear in this cytoplasm coarser granular bodies which stain heavily, and considerable plasmolysis is found here.

Pollen mother cells in the spireme and synizesis stages of *L. giganteum* show in the irradiated anthers another type of chromatin disturbance. In Figure 15 a section of an anther, exposed to 0.4 millicurie of radium emanation for 3 days, is seen. The chromatin has accumulated into one mass, to one side of the nucleus, while a ring of deeply stained fragments of chromatin material lines the nuclear membrane, thus giving the typical "signet ring" effect, shown by Grasnick ('18), for irradiated animal cells. The affected cells were apparently in the pachytene spireme stage. Some cells appear to be unaffected. The cytoplasm appears normal.

In another preparation exposed to 0.4 millicurie of bare radium emanation for 48 hours, the marked contraction of the nuclear material forms a homogeneously stained globular mass. These cells give the impression that they were in synizesis at the beginning of the irradiation. The chromatin material here also appears to have contracted, so that the nucleoli are squeezed out of their usual position in the chromatin knot. In some of the cells the chromatin seems to have exuded from the nucleus and to be contained in vacuolar globules scattered in the cytoplasm of the cell (Fig. 16). Membranes and clear areas are formed about them. Frequently a number of these areas appear to fuse to form larger bodies. Whether these are dwarf nuclei referred to above is questioned. It appears that they have formed subsequent to the irradiation. The control anthers taken from the same buds before the emanation was applied failed to show these bodies, although I am reporting below some further observations on chromatin extrusion, described by West and Lechmere ('15). The extrusion of chromatin material described by these authors generally extended to the adjacent pollen mother cell. Such is not the case here (Fig. 28). The cells, though round in shape, are in contact with each other on several sides. There appears to be no evidence of particles of material extending from the nucleus of one cell to the cytoplasm of its neighbor. It must be remembered that although the control anthers failed to show this chromatin exudation and what appear to be membrane formation about the particles, this phenomenon may occur in non-irradiated cells. While I noticed the different phases of the spireme and synizesis stages in normal, non-irradiated microsporocytes, it was almost impossible to be sure which stage was affected by the emanation, as I mentioned above, but that these stages are affected appears evident.

The effects of filtered irradiation were studied in spireme and synizesis stages. The exposures were made as in the other studies on buds. From 1 to 1.3 millicuries of radium emanation filtered through 0.5 mm. of silver and platinum was used. The exposures lasted from 1 hour to 48 hours with a lapse of 23 hours to 68 hours before the fixations were made. In all the irradiated anthers

studied, the coagulation and contraction of the chromatin material of the pollen mother cells was noticed.

Figure 17 represents a section through an anther of *L. superbum* exposed for $22\frac{1}{2}$ hours to 1.3 millicuries of radium emanation filtered through platinum, 0.5 mm. in thickness. The exposed anthers were removed 48 hours after the exposure was made. The pollen mother cells in this portion of the anther sac have not yet separated. The chromatin is coagulated and in most cases thrown to one side of the nucleus.

It appears that the injuries are as marked as in the non-filtered preparations reported above. The exposure, however, was much larger. Here also there is no uniformity in result. Some cells appear more injured than others. The exudation of the chromatin with extensions into the cytoplasm was not observed here. In another series of studies on *L. Harrisii* 1.1 millicurie of radium emanation filtered with platinum was introduced into buds for periods of 3, 5, 7, and 10 days. The exposures for 3 and 5 days gave the same results as I noted above. The longer exposures resulted in complete destruction of the microsporocytes in the immediate vicinity of the "seed" with slow degeneration in the remaining portions.

I am not convinced that these homogeneous masses of chromatin material described above play any further role in the development of the pollen mother cell as suggested by Koernicke ('05) for *L. Martagon*. It appears from my preparation that these cells die.

I have been unable to obtain definite data on the effects of the rays on cells in second contraction and diakinesis stages. These stages appear in abundance in control anthers studied. The progress of development from the pachytene stage to the formation of tetrads is rapid. Several stages in the development of the pollen mother cell may be observed in the same anther sac at the same time. Very frequently I have found in the control anthers pollen mother cells with the chromatin in the pachytene stage at one end of the locule, in another part, cells in diakinesis, while in still another part, the nuclei are already dividing. The remaining irradiated anthers from this bud show pollen mother cells with coagulated masses of chromatin. There is a breaking up of the pachytene spireme into more chromosomes than are generally found in the lily, as reported by Koernicke ('05).

The effect of the emanation on the reduction divisions was studied in a large number of buds in the 4 species of lilies. The anthers were exposed to 0.25–2 millicuries of radium emanation in bare glass capillaries for 1 hour to 72 hours. The anthers were fixed at frequent intervals. The results with the pollen mother cells in the heterotypic and homoeotypic divisions are the same. With short exposures definite effects are produced on the chromosomes on the spindle. In the development of the multipolar spindle, I have found the chromosomes clumped into a globular homogeneously stained body lying in the center of numerous radiating fibers which collect into 3 or more poles. Cells with multipolar polyarch spindles in which the nuclear membrane is still intact appear to be unaffected. Figure 18 represents a section of an anther which was exposed

to a bare seed of 0.4 millicurie of radium emanation for 48 hours. Figure 19 shows a part of a section of an anther in which the nuclei of the microsporocytes are in the bipolar spindle stage. The anther was exposed to 0.8 millicurie of radium emanation for 19 hours. The chromosome masses are in the metaphase and anaphase stages. The chromosomes have fused with each other into irregular bodies, and are unrecognizable as typical lily chromosomes. The staining power of these chromosomes is changed. Instead of a gentian violet coloration of the triple stain, the chromosome mass now takes on a homogeneous reddish color. Small globular bodies, probably chromatin fragments, are found on the spindle also.

In Figure 20, a slightly different stage in the effect of radium emanation on the chromosomes in the first division of the pollen mother cell may be seen. Here there is more evidence of fragmentation, yet the main mass of chromosomes has fused and spread over the entire spindle as if chromosomes in early anaphase stage had been interfered with. There is no evidence of the movement of the chromosomes to the poles in these irradiated cells. Yet the spindle fibers are apparently unaffected, although in many cases they seem to fuse, forming much thicker, fiber-like structures than are found in the normal control, shown in Figure 21. In Figure 22, a normal heterotypic division is shown in anaphase stage. Contrast the chromosome masses here with those in Figure 20. The photograph does little justice to my preparation.

The cytoplasm in these irradiated cells shows an abundance of extra-nuclear nucleoles, similar to those figured by Allen ('05). A study of these irradiated pollen mother cells shows that these extra-nuclear nucleole-like bodies are augmented in number by the chromatin fragments (Fig. 20). The cytoplasm of these irradiated cells is generally coarsely granular. Vacuoles appear in the cytoplasm of cells which are more markedly affected. As in the stages reported above, the effect of the raying is not uniform. Sections from irradiated anthers under similar conditions show preparations in which the entire spindle is covered with small bodies, most likely chromosome fragments. These stain diffusely, often obscuring the spindle fibers. With more intense irradiation, the chromosomes appear to be broken up into a number of coarse, granular bodies which are scattered through the cytoplasm. The spindle fibers stain faintly, and in many cases appear to be absent (Fig. 23). The cytoplasm in these cells appears plasmolyzed. In more severely affected cells, the cytoplasm appears to have contracted, presenting an appearance similar to that found in irradiated early prophase stages of the pollen mother cells (Fig. 11). These observations are in accord with the results of Koernicke ('05). These preparations emphasize the fact that the chromosomes are visibly the most susceptible to the influence of the rays studied. The exposures were all unilateral, yet there is no evidence of a path or direction of the emanation. This favors the contention that the effects of the rays are induced by subsequent chemical changes. It appears as if the nuclear membrane tends to modify the effects of these rays on the nuclear material. The evidence from my preparations seems to indicate that the cells with injured chromosomes do not divide, but slowly undergo disintegration.

The daughter nuclei after the heterotypic division have also been studied with various quantities of radium emanation for short periods. That these daughter nuclei result from irradiated chromosomes is questionable. It is more likely that they were in the anther sac at the time of the irradiation. The developmental changes which follow the onset of the first division are very rapid. Pollen mother cells in the heterotypic division may be found in the same anther sac with cells in which the second division has already been completed.

The change in the daughter cells following the first division appears in the nuclei. Figure 24 represents a section of an anther of *L. Harrisii*, exposed to 0.8 millicurie of radium emanation fixed after an exposure of 18 hours, 50 minutes. Several slightly different stages are to be noted here. The daughter chromosomes appear unaffected in some nuclei, while in others they are fused into homogeneous masses in which the stain is uniformly red. In these nuclei the nuclear membranes are not yet completely formed. The more advanced daughter nuclei are apparently less injured. Cell division is not visibly affected by this irradiation.

The homoeotypic division occurs simultaneously in the 2 daughter cells. Anthers irradiated with bare "seeds" containing 0.8 millicurie of radium emanation with exposures of 1 hour to 3 days, examined at intervals of 6 hours, show effects of the chromosomes in division very similar to those observed in the first division. Figures 25 and 26 show the effect of raying after 19 hours. Delayed chromosomes are frequently seen in this stage. In the irradiated tetrad nuclei, the "hour glass" effect appears frequently. More than 4 daughter cells were occasionally found in my preparations. They were also observed in the control anthers.

Pollen grains exposed to the rays show no uniform reaction. Some grains in the immediate vicinity of the "seed" appear to be entirely unchanged, while others show the characteristic clumping of the chromatin material.

Parallel studies of the effects of the division of the pollen mother cells were made with filtered radium emanation. Three millicuries of radium emanation were used and the exposures lasted for a period of 10 days. The examinations of the irradiated anthers were made at 1, 3, 5, 7, and 10 days. The same effects described for the unfiltered rays were observed here. For the longer periods of exposure the buds became slightly etiolated, especially in the immediate zone of the "seed." A microscopical examination of the anthers showed the complete necrosis of the tissue of the anther sac. Occasionally vestiges of pollen mother cells may be seen. The walls of the anthers are apparently alive. The nuclei of these cells consist of globular bodies of chromatin material. The cytoplasm is sparse, and starch grains are few in number.

A small number of buds which were exposed to 2 millicuries of radium emanation filtered through silver 0.5 mm. in thickness for 3 days, are of especial interest. The anthers showed a large number of well-formed, normal, pollen grains. Some of the grains showed characteristic clumping of the chromatin. Among these I have noticed a small number of grains which were disintegrating. Figure 27 represents a section through such an anther. One of the pollen grains has increased enormously in size. Its nucleus is partially disintegrated. Part of

the nucleus shows the clumping of the chromatin and is densely stained. The cytoplasm has become filled with fibrillar elements, while in another part of the cell the coarse granular structure still persists. The fibrillar portion of the cytoplasm appears to be in the disintegrating portion of the cell. The cell wall also shows evidence of breaking down. Whether these cells are products of irradiation I am unable to state definitely. None such appeared, however, in my control preparations.

West and Lechmere ('15), have described in *Lilium candidum* a type of chromatin migration which I have observed in all 4 of the species of lillies studied. These authors failed to learn the fate of the cells in which this phenomenon occurs.

In my preparations the migration of the chromatin material from the nucleus of one pollen mother cell to the cytoplasm of the adjoining cell occurs most frequently at the time of synizesis. Small globules of chromatin-like substance take part in this migration. The nucleus of the cell lies close to one wall and it is through this wall that the chromatin appears to enter the neighboring cell. The chromatin-like body in the new cell becomes surrounded by a clear space and a delicate membrane is formed on its periphery (Fig. 28). The migration of the chromatin continues. The cytoplasm becomes vacuolate and disintegration sets in. The recipient cell becomes filled with these granules and eventually disintegrates too. While a cell may receive the extrusions from one cell, it is giving off chromatin-like bodies to another. Cells which bear such migrating chromatin bodies, disintegrate. The conditions that favor the development of this type of cell I have been unable to learn. I have irradiated these pathological cells and find that with very short exposures and small doses of unfiltered radium emanation, rapid and complete destruction is brought about.

This work was undertaken in collaboration with Dr. Isaac Levin, Director of the New York City Cancer Institute. To him I am indebted for a large number of bare "seeds" used in this work. I am indebted to Dr. Muir, Radium Emanation Corporation, for some of the platinum screens.

Summary

1. A study was made of the effect of filtered and unfiltered radium emanation on the cells of the various stages in the development of the sporogenous tissue in the anthers of *L. Harrisii*, *L. giganteum*, *L. auratum*, and *L. superbum*.

2. The study stresses the effect of the emanation on the early stages in the development of the sporogenous tissue.

3. The radium emanation used in bare glass capillaries, varied in quantity from 0.25 millicuries to 3.3 millicuries. Filters of platinum, silver, and aluminum 0.5 mm. in thickness were used to screen the glass capillaries.

4. Fifty-two experiments were made in which anthers in all stages of development were exposed to bare and filtered glass capillaries with 0.25-3.3 millicuries of radium emanation for 1 hour to 10 days. These were prepared for microscopical examination at intervals of 12, 16, 19, 24, 48, and 72 hours, and 5 and 7 days.

5. Thirty-two experiments were made in which anthers at all stages of development were exposed to bare and filtered glass capillaries with 0.5–1.7 millicuries of radium emanation for 1–48 hours, at intervals of 1 hour for the first 4 hours, and then at intervals of 4 hours to 48 hours. At intervals of 12 hours, and up to 72 hours after exposure the anthers were removed and studied.

6. Parallel control studies were made. Buds of the same size and of the same species as those irradiated were fixed at the beginning of each experiment. Another form of control consisted of removing 2 or 3 anthers and the ovary from each bud to be used, before the capillary bearing the radium emanation was introduced. Empty metal tubes of the same size as those used to filter the radium emanation were introduced into buds of the same age as those treated with the radium emanation.

7. The effects of small doses of radium emanation on the nuclei, nuclear division, and cytoplasm in young cells of the sporogenous tissues of the anther are noted. The effects of filtered and unfiltered radium emanation on the principal stages in the development of the pollen mother cell, and especially the injury to the chromatin material in the various division stages, are described.

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DESCRIPTION OF FIGURES

Photographs were made with Bausch and Lomb D. and L. type camera, and Tungsarc lamps Zeiss' 8 mm. apochromatic objective and compensating ocular No. 18 were generally used. Figure. 3 and 5 were made with the aid of 16 mm. apochromatic objective and compensating ocular No. 18. Figure 24 was made with the aid of an 8 mm. apochromatic objective and compensating oculars Nos. 8 and 18. For Figures 4, 7, 8, 12, 21, and 26, a 1.5 mm. apochromatic objective and compensating ocular No. 8 were used.

Figures 1, 2, 4, 5, 7, 8, and 17 represent sections of *L. superbum*.

Figures 3 and 6 represent sections of *L. auratum*.

Figures 9, 15, 20, 21, 22, and 23 represent sections of *L. giganteum*.

Figures 10, 11, 12, 13, 14, 16, 18, 19, 24, 25, 26, 27, and 28 represent sections of *L. Harrisii*.

Fig. 1. Sporogenous tissue of young anthers exposed to 3.3. mc. radium emanation for 1 hour. Bellows 38 cm.

2. Section of sporogenous tissue of young anther control, exposed to empty metal tube for 1 hr. Bellows 36 cm.

3. Sporogenous tissue of young anther exposed to 0.49 mc. radium emanation for 24 hrs. Bellows 35 cm.

4. Enlarged portion of sporogenous tissue of young anther exposed to 0.7 mc. radium emanation for 24 hours. Bellows 40 cm.

5. Sporogenous tissue of young anther showing vacuolate cytoplasm, exposed to 0.9 mc. radium emanation for 33 hours. Bellows 36 cm.

6. Young sporogenous cells exposed to 1.7 mc. radium emanation filtered through 0.5 mm. thickness of platinum for 1 hour, fixed 24 hours, after the exposure. Bellows 40 cm.

7. Similar tissue exposed for 19¼ hours to 1.1 mc. radium emanation through 0.5 mm. thickness of platinum, fixed 73 hours after the exposure. Bellows 35 cm.

8. Sporogenous layer of anther exposed to 3.3 mc. of radium emanation for 1 hour. Bellows 32 cm.

9. Sporogenous tissue of same age as that shown in Figure 8; control, exposed to empty tube for 48 hours, shows dwarf nuclei. Bellows 29 cm.

10. Young pollen mother cells exposed to 0.4 mc. of radium emanation for 72 hours. Bellows 33 cm.

11. Section of anther from the same bud as that shown in Figure 10, in contact with glass capillary. Bellows 33 cm.

12. Pollen mother cells exposed to 1.1 mc. radium emanation filtered through 0.5 mm. thickness of silver for 7 days. Bellows 40 cm.

13. Normal spireme stage of the control anther. Bellows 39 cm.

14. Spireme stage of pollen mother cell anther exposed to 0.8 mc. of radium emanation. Bellows 40 cm.

15. Similar stage exposed to 0.4 mc. of radium emanation for 72 hours; shows "signet ring" effect. Bellows 33 cm.

16. Synaptic stage exposed to 0.4 mc. of radium emanation for 48 hours. Bellows 37 cm.

17. Another synaptic stage exposed to 1.3 mc. radium emanation through 0.5 mm. thickness of platinum for 22½ hours, fixed 48 hours later. Bellows 40 cm.

18. Multipolar and bipolar spindle stages of the heterotypic division exposed to 0.4 mc. of radium emanation for 48 hours. Bellows 34 cm.

19. An older stage exposed to 0.8 mc. of radium emanation for 18 hours, 50 min. Bellows 37½ cm.

20. Another stage of the first nuclear division with same exposure given the anther shown in Figure 19. Bellows 37½ cm.

21. Normal nuclear division of pollen mother cell in control anther. Bellows 26 cm.

22. Normal anaphase stage in control anther. Bellows 37½ cm.

23. Another section of an anther in which pollen mother cells were undergoing the first division, exposed to 0.4 mc. of radium emanation for 72 hours. Bellows 40 cm.

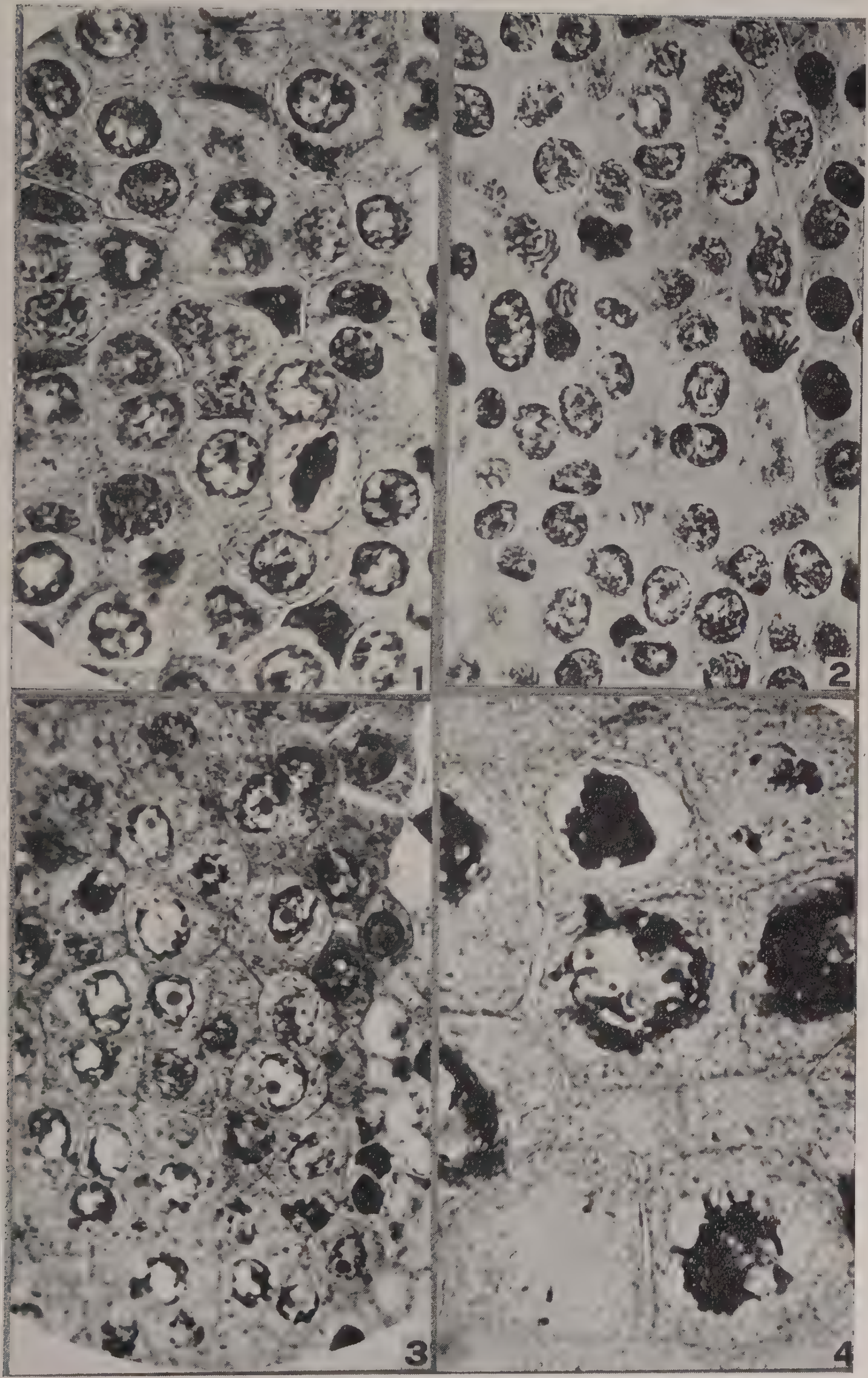
24. Pollen mother cell after first division, lower portion under higher magnification, from the same anther, exposed to 0.8 mc. of radium emanation for 18 hours, 50 min. Bellows 40 cm.

25. Homoeotypic division of nuclei exposed to 0.8 mc. radium emanation for 18 hours, 50 min. Bellows 37½ cm.

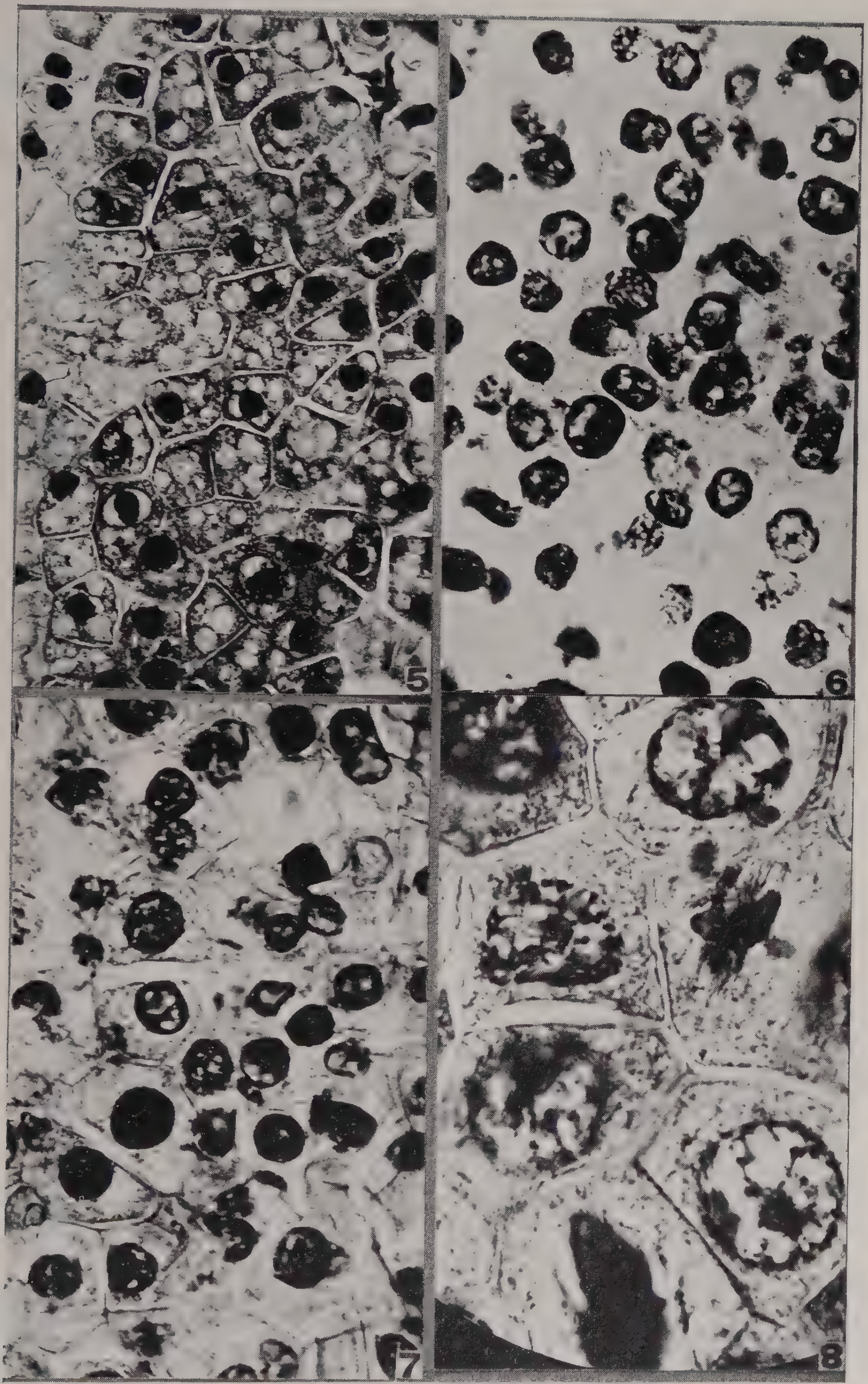
26. Enlarged view of cell shown in figure 25. Bellows 31½ cm.

27. Pollen grains exposed to 2.1 mc. of radium emanation, filtered through 0.5 mm. silver, for 76 hours. Bellows 40 cm.

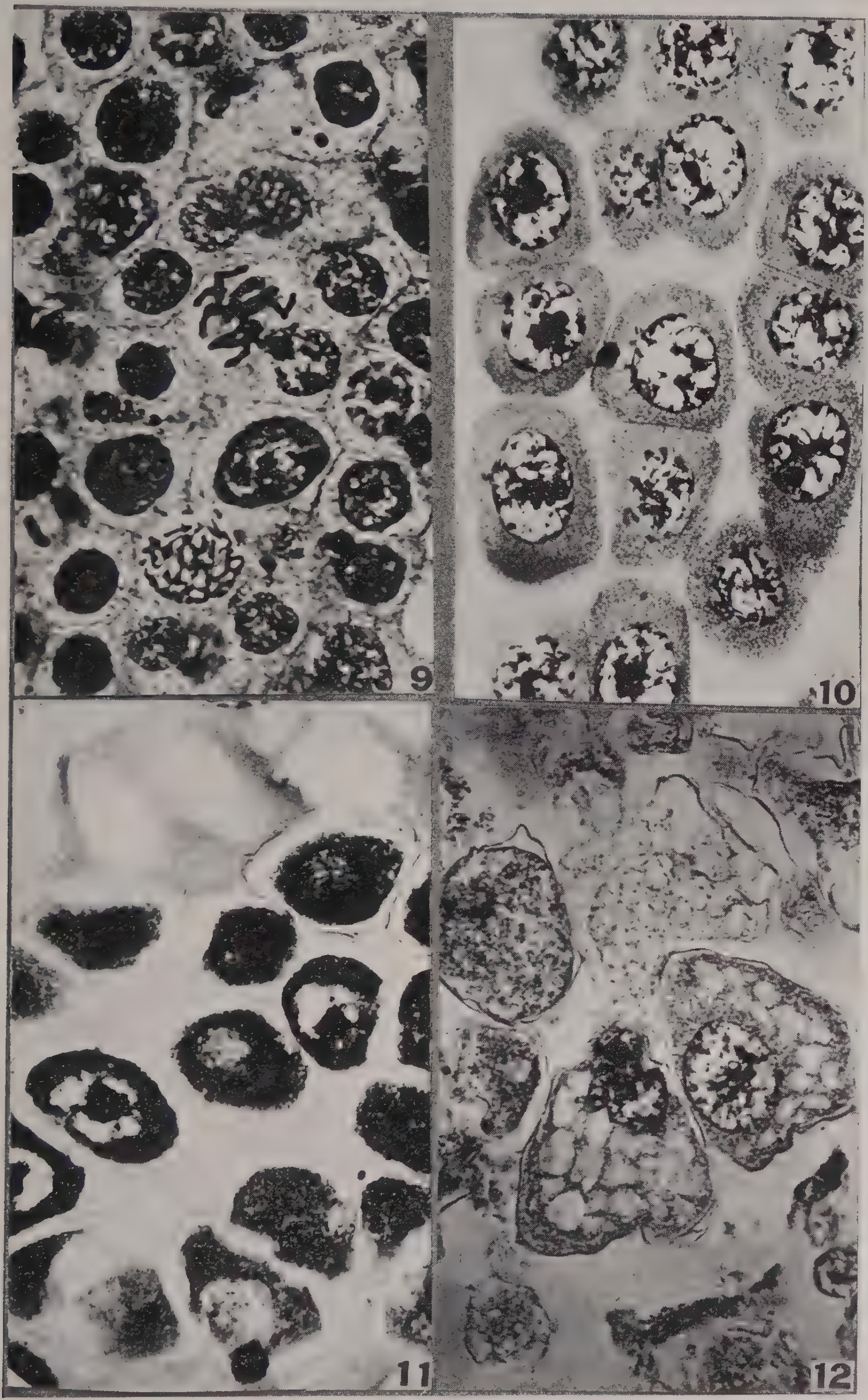
28. Synaptic stage showing migration of chromatin-like bodies from nucleus of one cell to cytoplasm of neighboring cell. Bellows 40 cm.



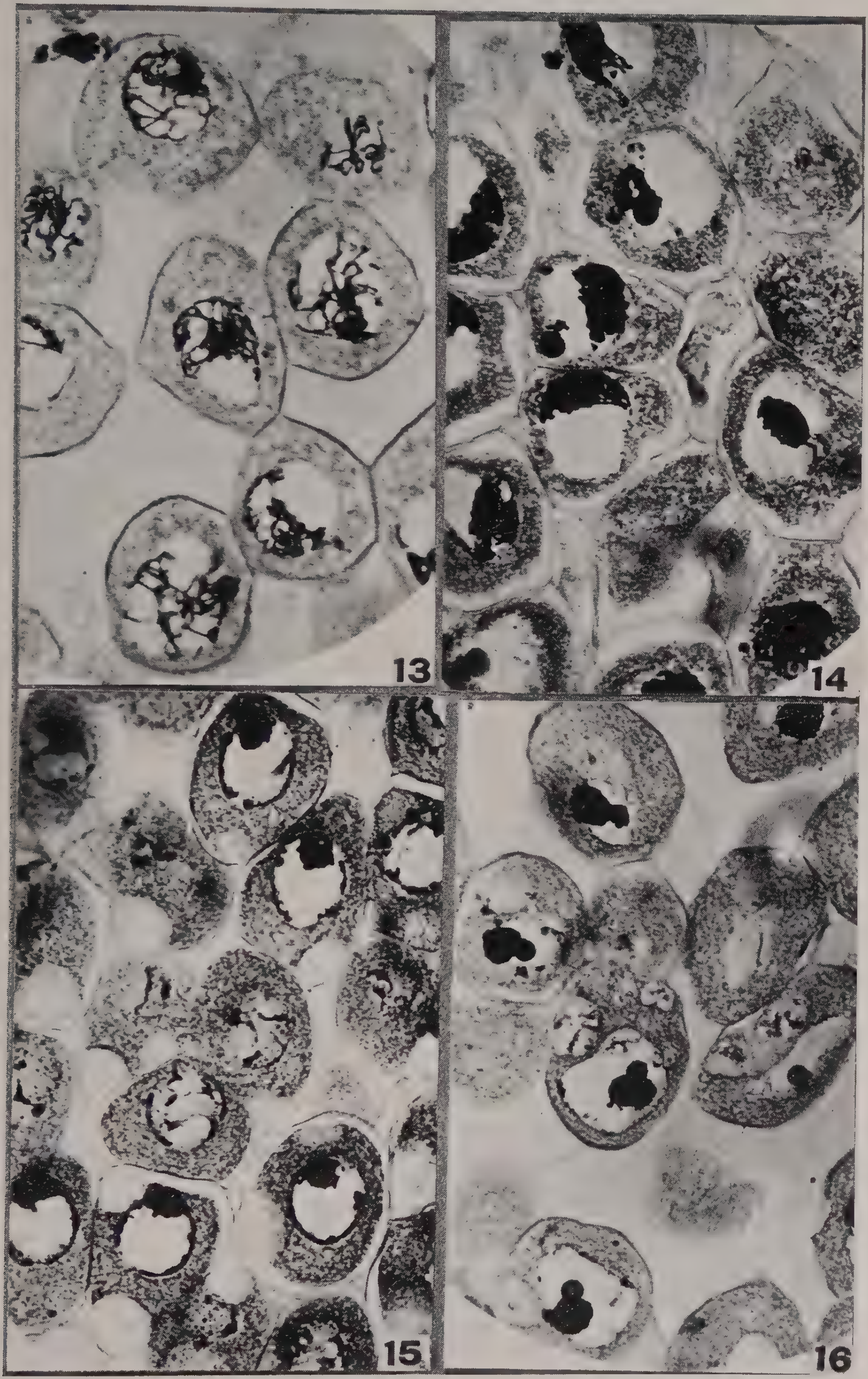
LEVINE: IRRADIATED TISSUES



LEVINE: IRRADIATED TISSUES



Levine: Irradiated Tissues



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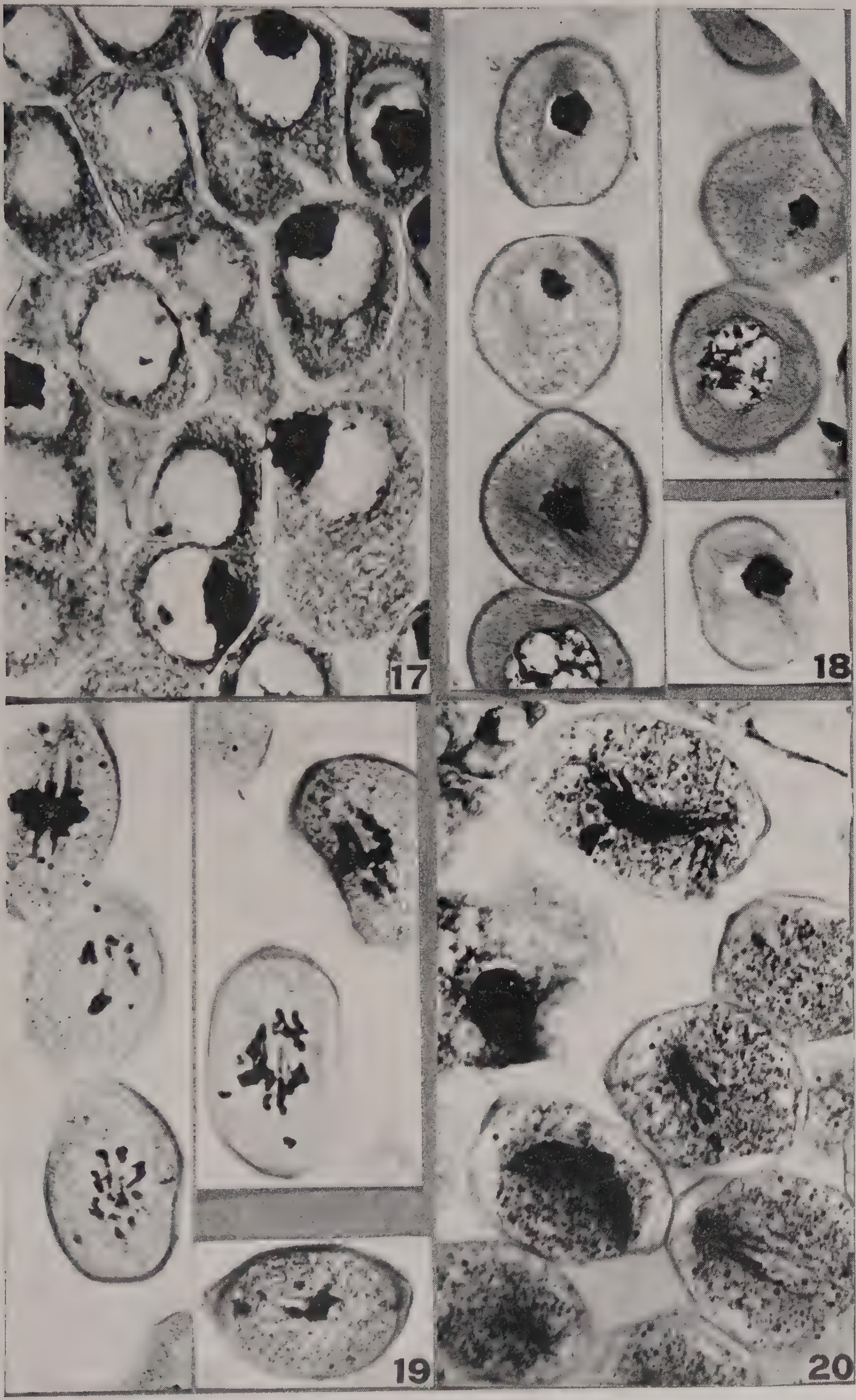

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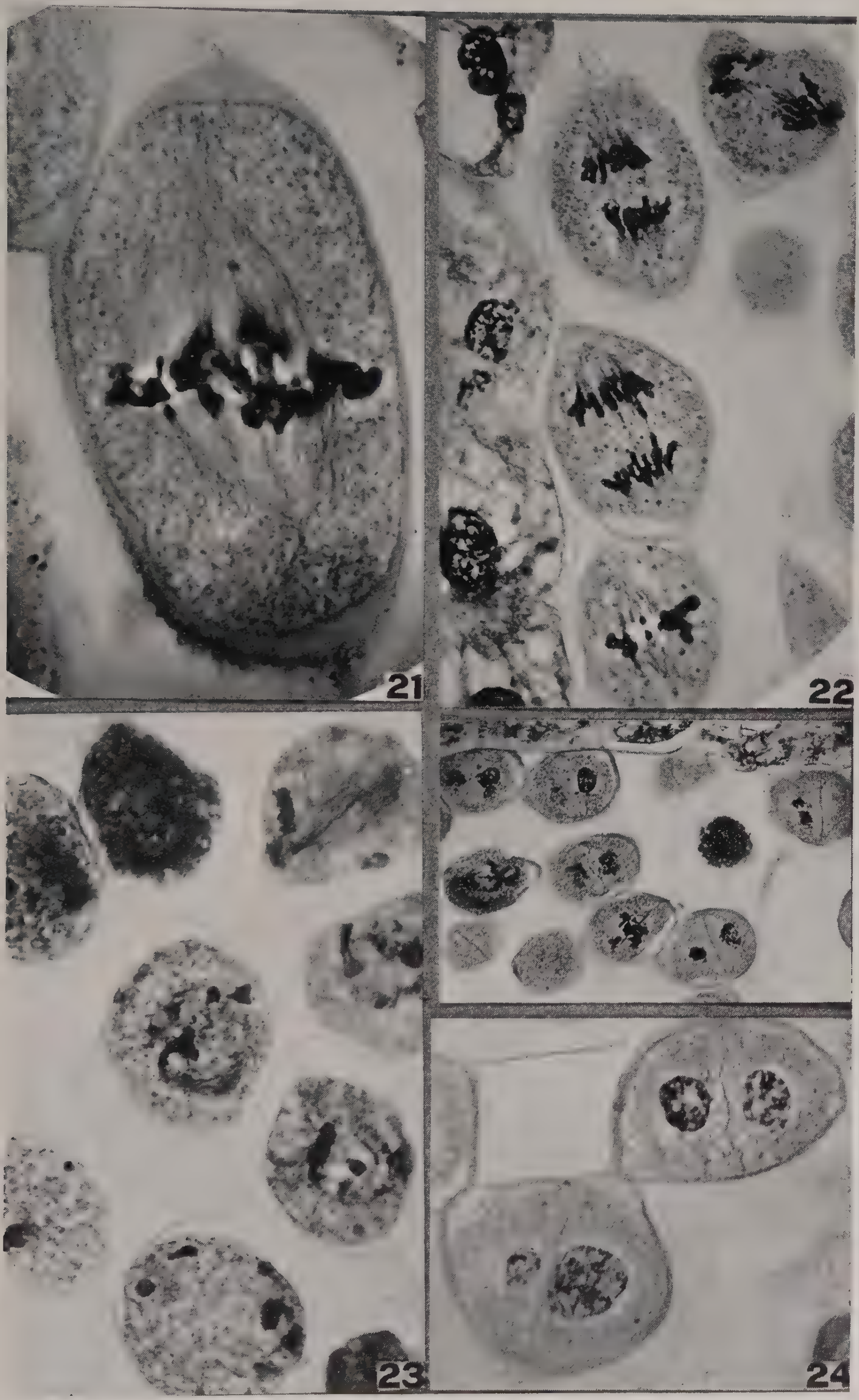

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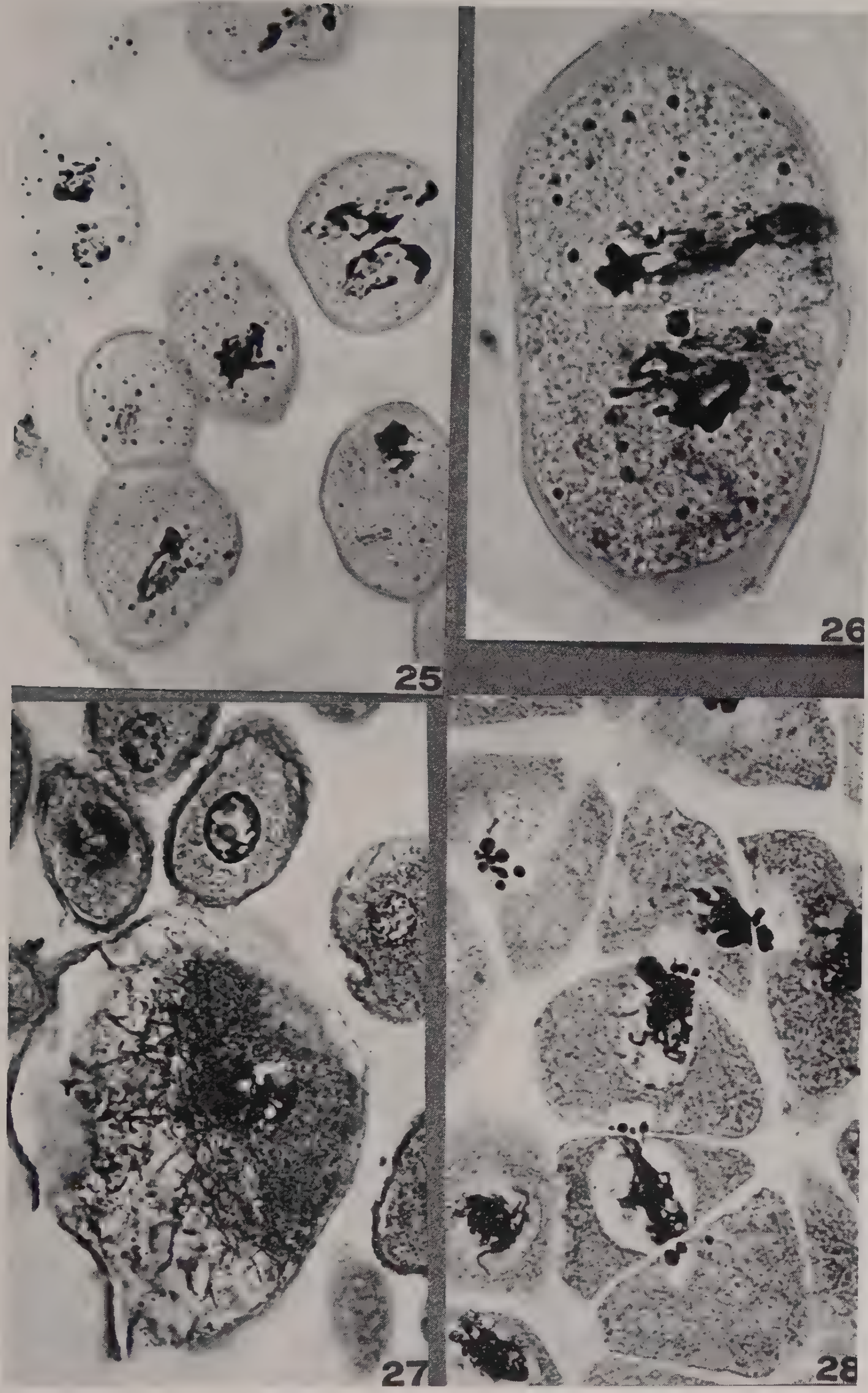

LEVINE: IRRADIATED TISSUES

ON THE OCCURRENCE OF SEX CHROMOSOMES IN FLOWERING PLANTS WITH SOME SUGGESTIONS AS TO THEIR ORIGIN[1]

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For a long time we have realized that the Mendelian type of inheritance is to be found in both animals and plants; the same applies to the correlated mechanism of chromosome reduction. In spite of this, although it has been known for sometime that in animals the sex of the individual may be definitely related to the distribution of special "sex chromosomes," these bodies have not been demonstrated in angiosperms until relatively recently. They were found by Allen in the gametophyte of the liverwort *Sphaerocarpos* some time ago and it seemed likely that they would occur in the diploid angiosperm sporophyte, which is more akin, cytologically speaking, to the diploid animal soma. On the other hand, the angiosperm plant is very frequently bisexual, that is to say, with both kinds of spore on the same plant and with flowers either hermaphroditic or unisexual; and it is only in strictly dioecious plants that one could possibly expect to find a mechanism comparable with that in the animal.

When Dr. Harrison and I, in a paper read at the meeting of the British Association for the Advancement of Science at Hull in September, 1922, described what we thought to be sex chromosomes in *Populus tremula*, we did not like to be too dogmatic about the question, for, although a definitely unequal pair of chromosomes was described in the male meiosis in the anther, its members were of such a size that it would be difficult to be sure of identifying a corresponding equal pair in the female. We should have hesitated less at the present stage, since more straightforward cases have now been described in which sex chromosomes have been identified in both sexes. Still, I think it should be carefully borne in mind that unequal pairs of chromosomes have been described by Carothers and others which were due to the crossing of races wit h slightly different chromosome complements, and these have thus no relation to sex at all.

To return to the historical side of the question, we find that in March, 1923, Santos published a paper describing an unequal pair of chromosomes in the staminate plant of *Elodea gigantea* but not until later did he describe the pistillate plants. The first accounts showing the conditions in the two sexes were those of Kihara and Ono on *Rumex acetosa* and my own on *Lychnis* (*Melandrium*) *alba*. In the same year Winge published an account of sex chromosomes in the stami-

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nate plants of *Humulus*, *Melandrium*, and others. Since then they have been described in a considerable number of forms, most of which are listed in Table 1. Negative records such as that of Yampolsky for *Mercurialis annua* need not disturb us, since these are again in accord with the findings in animals.

TABLE 1. SHOWING CONDITION OF CHROMOSOMES IN CERTAIN STAMINATE AND PISTILLATE PLANTS

Species	♂ Plant	♀ Plant	Authority
Populus tremula	36+X+Y	36+2X	Blackburn and Harrison
" trichocarpa	"		Meurman
" balsamifera	"		Meurman
" Simoni	"		Meurman
" serotina	"		Blackburn
" Eugenei	"		Blackburn
" generosa (etc.)	"		Blackburn
Salix viminalis	"		Blackburn and Harrison
" " var. yezoensis	"		Sinoto
" japonica	"		Sinoto
" cinerea	74+X+Y		Harrison
" aurita	"		Harrison
" Andersoniana (etc.)	112+X+Y		
Rumex acetosa	12+X+2Y	12+2X	Kihara and Ono, Sinoto
" thyrsiflorus	"	"	Meurman
" acetosella	38+2X+Y		Meurman, Kihara
Melandrium album	22+X+Y	22+2X	Blackburn, Winge, Meurman, Heitz
" rubrum	"	"	Blackburn, Winge, Meurman, Heitz
" album×rubrum	"	"	Blackburn
" glutinosum	"	"	Blackburn
" divaricatum	"	"	Blackburn
Silene Otites	"	"	Blackburn
Humulus Lupulus	18+X+Y		Winge
" japonicus	"		Winge
Cannabis sativa	"		Hirata
Urtica dioica	46+X+Y		Meurman
Morus bombycis	13+X+Y		Sinoto
Trichosanthes japonicus	10+X+Y		Sinoto
Valeriana dioica	14+X+Y		Meurman
Elodea gigantea	46+X+Y		Santos
" canadense	46+X+Y	46+2X	Santos
Vallisneria spiralis	16+X		Winge
Dioscorea sinuata	34+X		Meurman

The considerable body of positive records collected in so short a time is on the whole more consistent than the reports for animals; in all cases the male is the heterozygous sex. The actual type of chromosome mechanism may be classified, according to the condition in the male, in three groups:

I. The X chromosome accompanied by 2 smaller Y chromosomes; for example, *Rumex acetosella*.

II. The X chromosome with a smaller Y partner; for example, *Elodea*.

III. The X chromosome without a partner; for example, *Vallisneria*.

In connection with the third group there seems to be considerable doubt. In *Dioscorea* Meurman himself suggests that the evidence is insufficient. With regard to *Vallisneria*, Winge did not obtain heterotypic divisions, and the curious linking up of the chromosomes in the pollen grain division would seem to make observation at this point very difficult. In addition to this, Mr. W. C. F. Newton

tells me that the somatic chromosome number for both sexes is 20, which is not in agreement with Winge's figure. In view of this I think we must consider the case for the occurrence of the Protenor type of mechanism as not yet fully established.

The first, or XYY type, is so far limited to *Rumex*, and it may possibly be connected with the typical XY form through *Rumex acetosella*.

The plants which I, personally, have worked on, all fell into the second group, the "*Lygaeus* type" of the zoologists.

In *Populus* the cytological features are extremely constant throughout the genus, any variability being limited to the actual size of chromosomes. In all species and hybrids examined there are 19 pairs of chromosomes of assorted sizes and the heterochromosome pair consists of a medium-sized and a small member. The chromosomes are very small and thus very difficult to study, hence I am able to state with regard to the female only that all the pairs appear to be equal.

The Salicaceae are a dioecious family and the sex relations are fairly definite, although, even here, they may be disturbed under the influence of hybridity or the attack of certain parasites.

In a dioecious family of this type there is little hope of getting any idea of the origin of the two sexes, much less of that of the sex chromosomes. On the other hand, a group showing varieties of sex form has at least better possibilities in this respect; hence I would direct your attention for a little while to the section Lychnideae of the Caryophyllaceae. In this group are to be found an astonishing range of forms even within the limits of a single genus. The genera are, however, so closely allied that we shall consider the group as a whole and arrange the sex forms in a logical series. At the one end of this series are to be found many of the small-flowered annual forms, such as *Silene Behen* or *S. Bergiana*, which are hermaphrodite and habitually self-pollinated. *S. mekinensis* is strongly proterandrous, and thus cross-pollinated though still hermaphrodite. This stage is perhaps even better illustrated by *Heliosperma alpestre* in which the stigmas are practically invisible when the flower first opens, and only when the stamens are quite dead and reflexed do the stigmas grow to a quite considerable size. Proterandry is typical of the hermaphrodite flowers of the rest of this series, of which *S. italica* forms the next link in the chain. Here the first-opened flowers, the terminal ones of each branch, are female, whereas the later ones are all hermaphrodite. This condition probably has some definite cause of a physiological nature, since the stamens of the female flowers do develop to a certain stage. Germane to this is an observation on *Viscaria Sartori:* last year a perfect reduction division took place in the stamens, but when the flowers opened they were female with only the veriest relics of stamens; this year the same plant is completely hermaphrodite. Collins records a similar change from female to hermaphrodite in *Silene nutans*. In *S. inflata* some plants show the condition described for *S. italica* whereas others are definitely either female or hermaphrodite. The forms described for *S. nutans* are of similar variety. In *S. acaulis* again we have a form with diverse types in which the various races behave differently. It is commonly considered to be dioecious, though hermaphrodite

forms are described from Switzerland. I myself have observed "male" plants from the Rocky mountains bearing a few capsules late in the season, and hermaphrodites from Switzerland in which the late flowers are female. This seems to suggest that local food or water-supply within the plant might have an influence on the sex of the flower produced. This might be correlated with the change of sex in broken branches recorded, for instance, by Schaffner and by Harrison. In *S. acaulis* there is thus a suggestion of opposed female and male types, but the condition is not by any means stereotyped. *S. Otites*, although described in some of our floras as sub-dioecious, is much nearer the dioecious condition. Mr. W. C. F. Newton tells me that he has a male plant on which a female branch bearing fertile seeds has appeared; nevertheless, the plants are normally dioecious, since cutting the ovary of the apparently hermaphrodite flower reveals the fact that it is completely destitute of ovules. The dioecious species of *Melandrium*, commonly called *Lychnis*, complete the series of sex forms in the group.

Comparative studies of the group in respect to sex lead one to suggest that the original ancestor was of a somewhat indefinite polymorphic type and that from it have developed in different lines the hermaphrodite forms and the definitely dioecious forms, the latter possibly arising *via* a trioecious condition. Now the question arises: Is the definite alternation that is to be observed in the dioecious organism the result of the specific sex chromosome mechanism, or is it a mere coincidence that they occur together? A cytological investigation of the group is being made in order to try to throw light on this question. The dioecious species examined all show sex chromosomes: this statement applies to several dioecious forms of *Melandrium* and *Silene Otites*. On the other hand I have so far failed to find any such structures in other species, though they might conceivably occur in gynodioecious forms.

The reduced chromosome number throughout the group is typically 12; among about 30 species examined only 3 were found to be tetraploid. In the late diakinesis the bivalent chromosomes are ring-shaped (Pl. II, Figs. 4, 7, 8) though this form is less well marked in the dioecious species of *Melandrium* than elsewhere. At this stage also the bivalents seem still to be connected to one another by linin threads (Pl. II, Figs. 7, 8). To the subject of this phenomenon I shall return later.

In the majority of the species the ring-shaped bivalents are all approximately equal in size. The dioecious forms and one other species differ in this respect. The latter is *Silene viridella* Pursh (a form of *S. nutans*) in which 1 of the ring-shaped bivalents is approximately twice the size of the others (Pl. III). This does not appear to be the case in the typical *S. nutans*.

The phenomena in the dioecious species need more careful description. Of the genus *Melandrium* the following dioecious forms have been examined: *M. album*, type and var. *glabrum*, *M. rubrum*, the hybrid *M. album*×*M. rubrum*, *M. divaricatum*, and *M. glutinosum*. All these forms are strikingly similar in their cytology(see Pls. IV, V). The sex pair is larger than the other 11 bivalents. In the male this pair consists of elements of different size, and the larger member

is apparently somewhat double in shape, though not so markedly so as in the case of the *Humulus* figured by Winge (Winge '23, Blackburn '24).

The large bivalent in the female is ring-shaped and consists of equal members similar in size to the larger member of the XY pair in the male, hence the larger chromosome in the latter pair is the X. In my original description I put forward the opposite opinion, but since Meurman and Heitz have described the sex pair in the female as ring-shaped, I have re-examined my original preparations and have come to the conclusion that I was unfortunate enough to see it always in profile view and was thus deceived as to its size. New preparations in six different races have revealed the ring shape clearly (Pl. V, Figs. 6–12). *Silene Otites* is a very polymorphic species and I have been able to examine four different races of it. Most of the work has been done on the two sexes of material which I obtained in Switzerland while working at Professor Chodat's Summer School last year. Further observations have been made on the female of a strain from Kew and on preparations from the male of material from Norfolk and from a very different form from Bulgaria, both of which were kindly lent to me by Mr. W. C. F. Newton of the John Innes Horticultural Institution.

The cytology of this species is particularly difficult; the fixation is capricious, so that ideal figures are rather scarce, and the very close packing of the chromosomes at the metaphase makes their outlines rather difficult to observe. In view of this, the description below must be considered as somewhat provisional, though the result of the closest investigation of the material in hand. In good sections in late diakinesis the bivalent rings are seen to be still linked to one another by fine linin threads, as described for the general type (cf. Pl. II, Figs. 7, 8; Pl. III, Figs. 3; and Pl. VI, Figs. 1, 7).

My first impression on looking at the material was that the sex chromosome group in the male was exactly of the type described for *Rumex acetosa*, with a small chromosome at either end of a U-shaped larger one, and I found, literally, dozens of figures which seemed to bear this out. Finally, however, I determined that the element at the one end really belonged to the next bivalent and was attached to the U chromosome by the long continuation of the linin connections described above. The X chromosome was thus pulled out into a U shape, even at the metaphase, instead of being retracted as in *Melandrium*. The Bulgarian material does not show this peculiarity quite so markedly, being in this respect half way between the typical *S. Otites* and the *Melandrium* type (Pl. VI, Fig. 6).

The behavior of the female is similar to that in *Melandrium*, since the XX pair is again ring-shaped.

The difficulty found by Meurman and Kihara with *Rumex acetosella* was probably due to a similar linking up of the chromosomes to that described above, and possibly further investigation on this line will clear up the discrepancy between the different accounts.

Cleland has described connections between the chromosomes which continue to the metaphase in species of *Oenothera*, and these have a profound influence on the heterotypic division. From my observations on *Silene*, it seems probable

that the occurrence of ring-shaped bivalents at the metaphase is actually due to long-continued linin connections between the bivalents (Pl. II, Fig. 8). It is possible that this hypothesis may have more general application.

To return to the question of the sex chromosome mechanism and its origin in the Lychnideae. If we compare the heterotype chromosomes of the female plant of *Silene Otites* with those of the pollen mother cells of S. *viridella*, we see that in each case there are twelve bivalent rings of which one is considerably larger than the rest. On the other hand, in the male of *S. Otites* the large bivalent is made up of one large member and one small one. This unequal pair is characteristic of the male plant of dioecious forms and might have arisen in various ways. In this group, however, the evidence seems to suggest that it owes its origin to crossing between a form like *S. viridella* and a normal type with all its chromosome pairs of equal size. To make the idea clear, let us imagine that *S. viridella* crossed with typical *S. nutans*. Then the F_1 plant would have a chromosome constitution similar to that of the male of *S. Otites*. If the F_1 were then back-crossed on *S. viridella*, the next generation would consist of equal numbers of individuals with an "XX" constitution and individuals with an "XY" consitution, that is, with exactly the mechanism characteristic of a dioecious species, and I should like to hazard a guess that they would actually be dioecious.

I hope to be able to make crosses next year to determine if it is possible actually to produce an XY mechanism in this way, and, if so, whether the sex exhibited will change simultaneously to a dioecious condition. Even if I fail to make the cross, there still remains the probability that the modern dioecious forms arose in some such way, since it is obviously impossible to repeat the exact conditions experimentally.

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Explanation of Plates

Illustrating the types of Chromosome complement found in the meiosis of the plants of the Lychnideae (Caryophyllaceae) Plates I and II show the typical condition, Plate III an unusual condition, Plates IV and V include figures from dioecious forms showing sex chromosomes. Drawings are from pollen mother cells unless otherwise stated.

PLATE I

Figs. 1–3 *Melandrium* sp. Figs. 4–6 *M. noctiflorum.* Fig. 7 *Lychnis Flos-cuculi.*

Fig. 1. Polar view of heterotype metaphase showing 12 bivalents.

Figs. 2 and 7. Equatorial view of the metaphase.

Fig. 3. Stage immediately preceding the metaphase showing linin connections still present between the bivalent rings.

Fig. 4. Polar view of the anaphase plate.

Figs. 5 and 6. Early anaphase side view.

PLATE II

Figs. 1–4. *Silene acaulis.* Figs. 5–7 *Silene antirrhina.* Fig. 8 *Viscaria sartori.*

Figs. 1 and 5. Polar view of the metaphase showing 12 bivalents.

Figs. 2, 3, and 6. Side view of the metaphase.

Figs. 4, 7, and 8. Diakinesis; 7 and 8 show linin connections between the bivalents.

PLATE III

Figures from *Silene viridella* showing 1 bivalent ring much larger than the remaining 11.

Figs. 1, 2, 6, and 7. Metaphase, equatorial view.

Figs. 3 and 4. Diakinesis showing linin connections in 3, but large ring not closed; 4 shows a more typical, closed ring.

Fig. 5. Anaphase showing large chromosome at each pole.

PLATE IV

Figures from *Melandrium divaricatum.*

Figs. 1–4. Heterotype metaphase showing large XY pair.

Fig. 6. Polar view of the same. Sex pair at S.

Fig. 5. Somatic anaphase showing unequal large sex chromosomes.

PLATE V

Figs. 1–5. *Melandrium glutinosum* male.

Figs. 6–12. From the female of as many different races of dioecious *Melandrium.*

Figs. 1 and 5. Anaphase showing sex pair at S.

Figs. 2–4. Sex chromosome pair from the metaphase.

Figs. 6–7 and 9–12. Metaphase and early anaphase figures to show the large XX pair in the form of a ring (S).

Fig. 8. First division of the embryo sac from two sections showing X chromosomes to the left.

PLATE VI

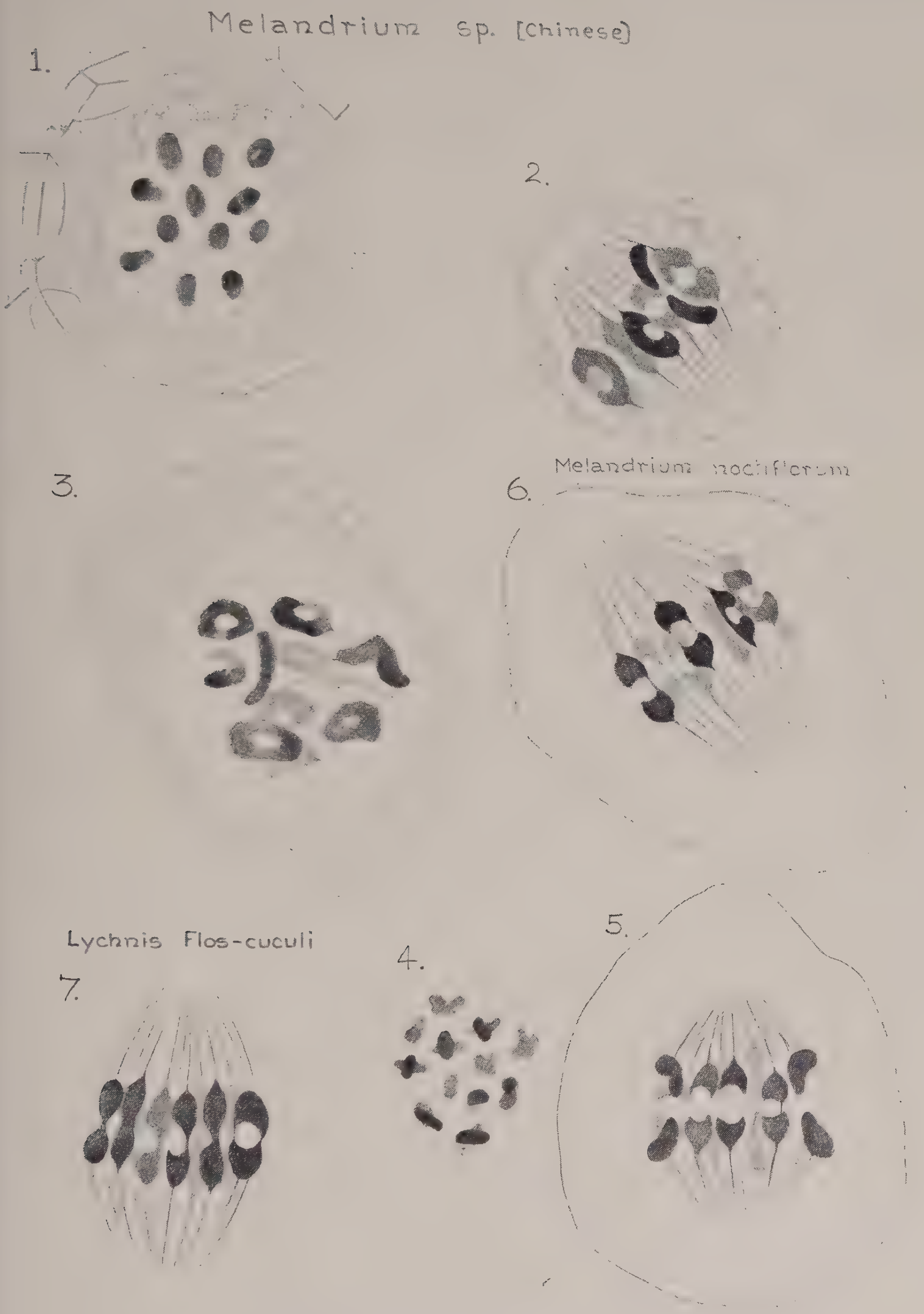
Figures from *Silene Otites.* 1–6 male. 7–9 female.

Figs. 1–3. Diakinesis, XY pair at S.

Figs. 4–6. Heterotype anaphase with sex pair at S.

Fig. 7. Diakinesis.

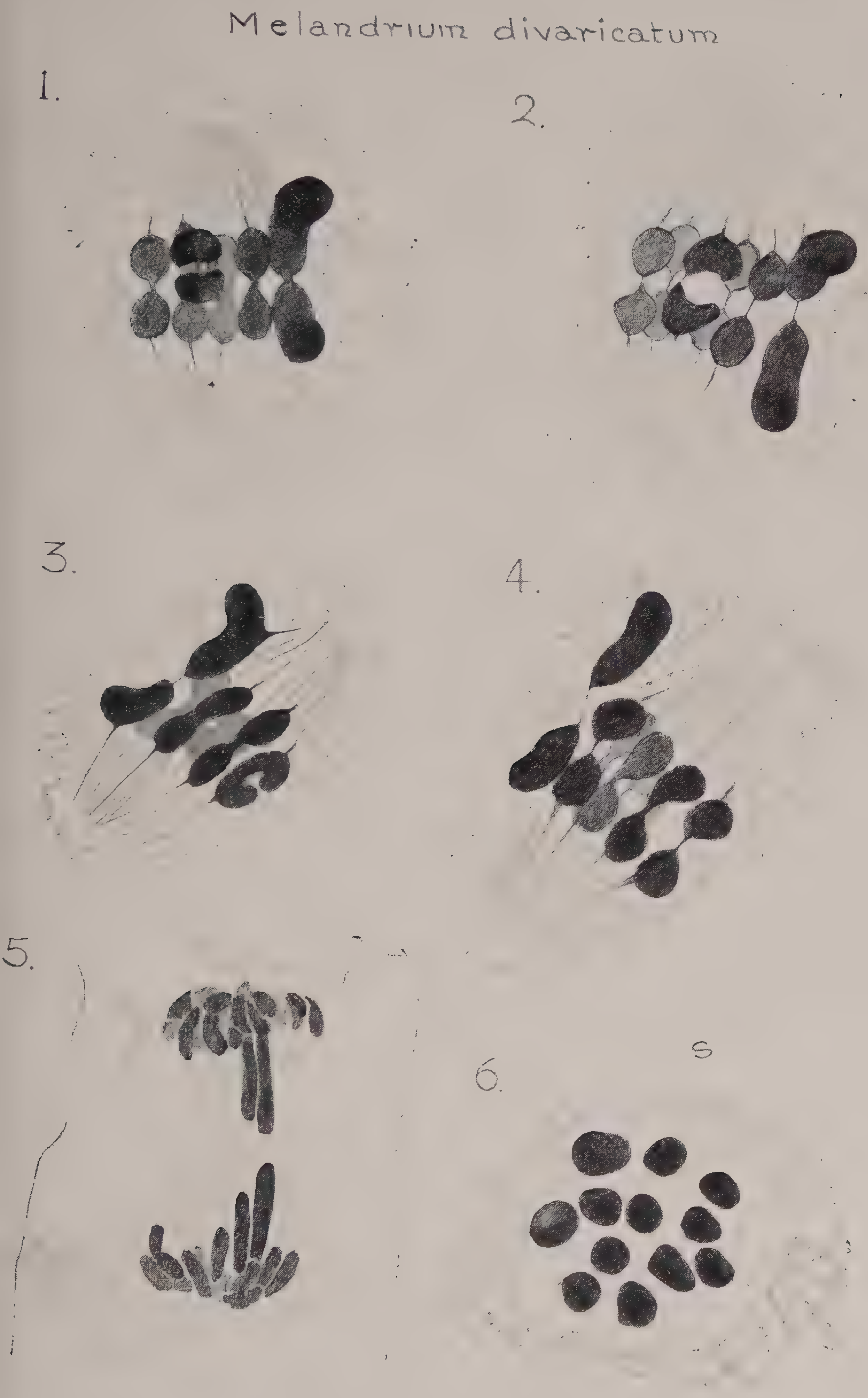
Figs. 8–9. Heterotype metaphase XX pair shown at S.



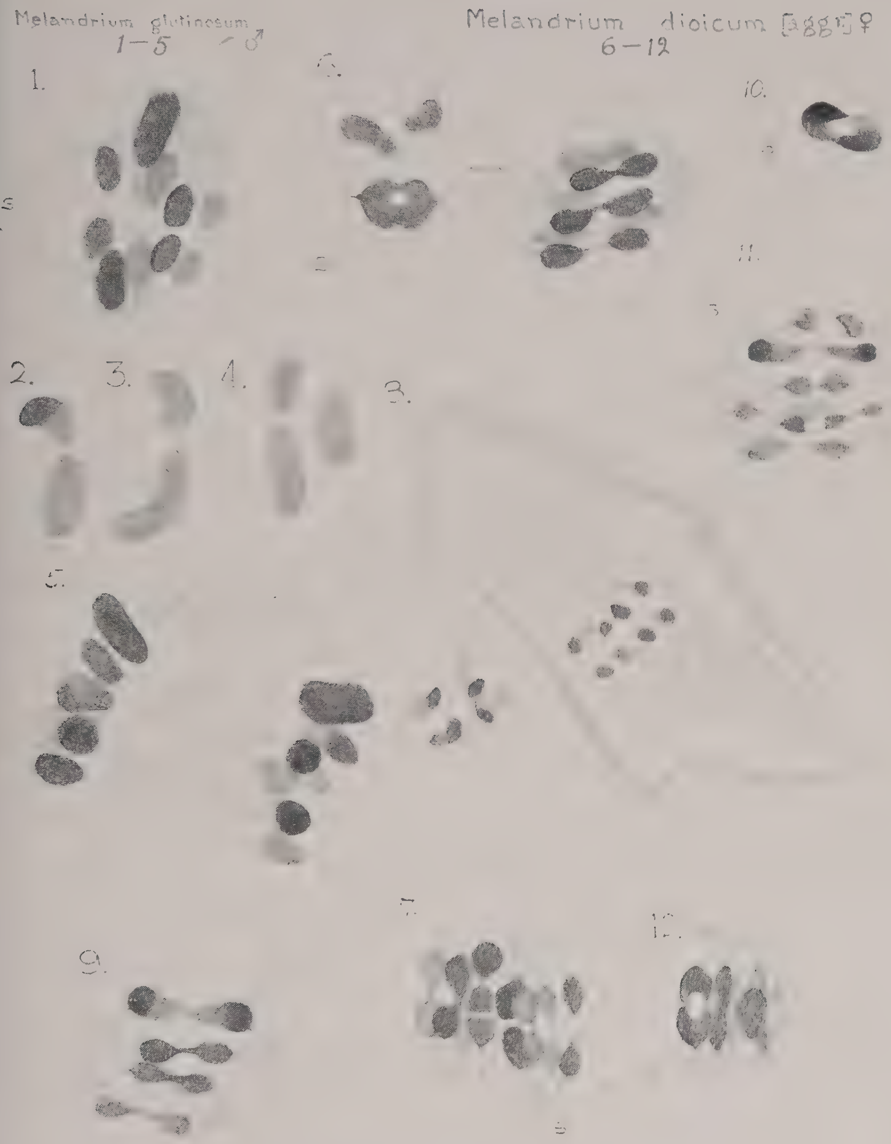
BLACKBURN: SEX CHROMOSOMES IN PLANTS

Blackburn: Sex Chromosomes in Plants

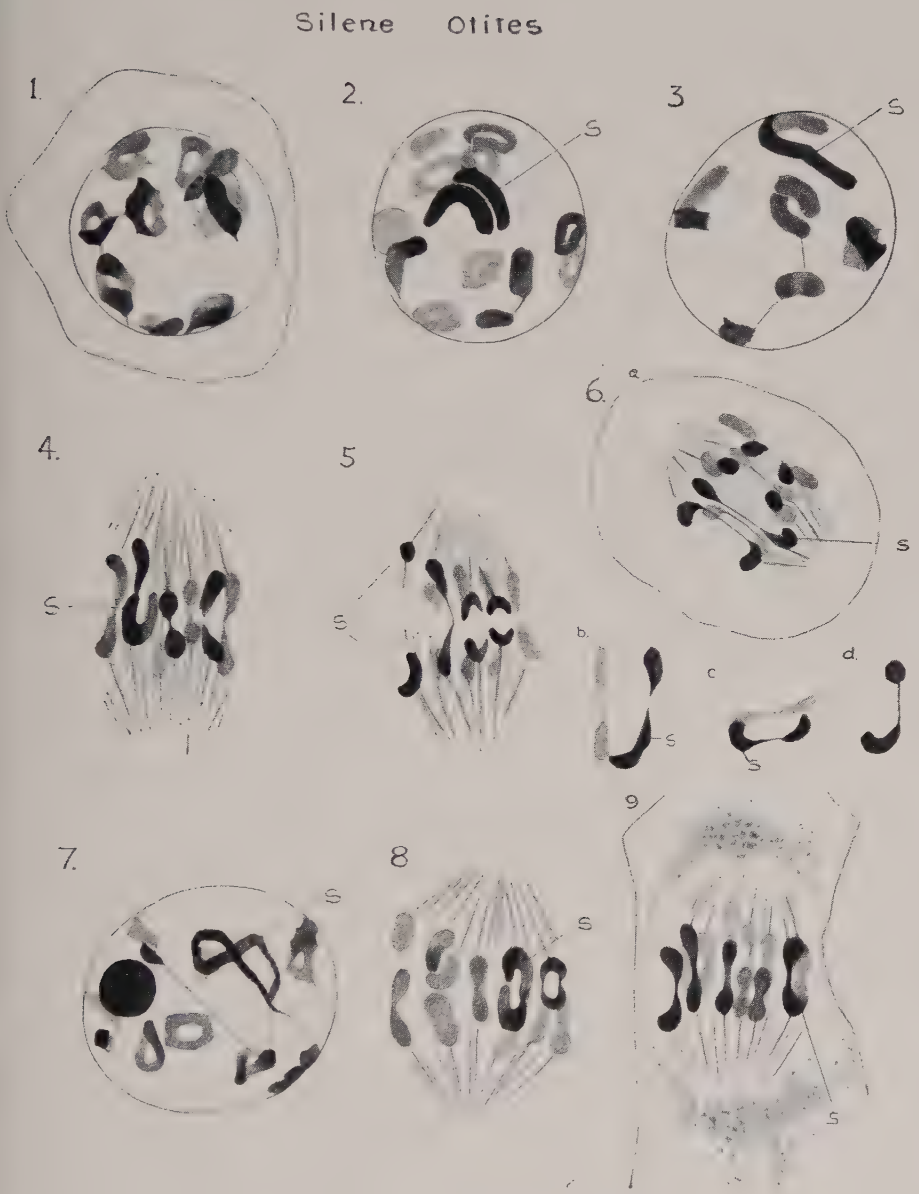
BLACKBURN: SEX CHROMOSOMES IN PLANTS



BLACKBURN: SEX CHROMOSOMES IN PLANTS



Blackburn: Sex Chromosomes in Plants



BLACKBURN: SEX CHROMOSOMES IN PLANTS

CHROMOSOME NUMBERS AND TAXONOMY[1]

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That chromosome numbers may be of great value in taxonomic studies appears from several recent investigations. For studies on larger systematic units than genera our present knowledge of chromosome numbers is not sufficient, but for investigations on the natural grouping and the relationships of the species within a genus they may be of importance. Compared with other morphological characters they have the great advantage that they may be more objectively judged. As carriers of hereditary substance or genes they must, furthermore, be regarded as of special importance in all species studies. Attempts to use chromosome numbers in taxonomic studies may be done in 2 directions: one may investigate the distribution of the chromosome numbers in the different systematic sub-groups of a genus with the purpose of eventually making, with the aid of these numbers, some corrections in the grouping of the species; or, one may use the numbers to test the limitations and the taxonomic value of the species of a *critical* group. In the latter case the question may be either to find out if a species is a unit, or if it consists of several types with different chromosome numbers, or to investigate if 2 nearly related forms are also to be distinguished from each other cytologically. Often spontaneous hybrids may also be detected cytologically. Studies of the kind suggested offer a new method for taxonomic work and, at the same time, they give a possibility of testing, cytologically, the reliability of current taxonomic working methods. They are, further, of great value for studies on species-formation and phylogeny in polymorphic genera.

As the cytological investigation of plant genera proceeds the result seems to be confirmed over and over that most plant genera have multiple chromosome numbers. At least, such numbers have been found in a considerable number of plant genera. In several cases it has been found that species belonging to the same section of a genus have all one and the same number, whilst different groups have different numbers. Thus, in *Triticum*, Sakamura and Sax have found the haploid numbers 7, 14, and 21, the 7-chromosome species being *T. monococcum*, the 14-chromosome species belonging to the Emmer wheats and the 21-chromosome species belonging to the Spelt wheats. In *Rosa*, Täckholm states that all species of Caninae are apomictical hybrids with 7 bivalent and 14, 21, or 28 univalent chromosomes in meiosis, whereas the other sections contain species with a regular reduction division and 7, 14, 21, or 28 gemini. Some of the last

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mentioned sections have only species with 7, or only those with 14 chromosomes, others contain both 7- and 14-chromosome species and Cinnamomeae consist of species with all four numbers. In the genus *Senecio*, Afzelius has found the haploid number to be 10 in the section Obejacoideae; 20 in Obejaceae, Jacobaea, Crociseris, and Ecalyculati; and 25 in Tephroseris. In recent work on *Brassica* by Shimotomai 5 species, belonging to 1 group, are reported with 10 haploid chromosomes, 2 of another group are reported with 18, and 1 of a third group with 9 chromosomes.

In some other genera another kind of relation between taxonomy and chromosome numbers has been found. In these cases different species-groups are distinguished by different, generally multiple, number-series, closely allied species thus belonging to the same series. Instances of this type are *Lactuca* and *Campanula*. In the former genus Ishikawa found the haploid number 9 in the subgenus *Lactuca* (in a limited sense), 5 in *Crepidiastrum* and *Paraixeris*, 7, 8, 12, 16, and 24 in *Ixeris*. In *Campanula*, Marchal found 10, 17, 34, and 51 in the section Medium; 8, 10, 13, and 17 in Rapunculus.

In *Crepis* Babcock and Mrs. Lesley have recently found some interesting relations between chromosome numbers and, especially, sizes and the taxonomic position of the species investigated. The authors also use cytological evidence, together with certain morphological characters, for a tentative rearrangement of the species. Also in a few more genera similar relations between chromosome numbers and taxonomy seem to exist (for example, in *Trifolium* or *Primula*), but the data as yet available are in these cases too incomplete to permit of any safe conclusions.

From my own investigations on *Carex* it is evident that this genus has irregular, not multiple, chromosome numbers. I have found at least 22 different numbers, ranging from 9 to 56. Attempting to find relations between chromosome numbers and taxonomic position of the species, I selected all those sections of the genus of which at least 2 species had been investigated cytologically, and arranged them in the following table (published in my paper of 1924).

TABLE 1. SYSTEMATIC DISTRIBUTION OF THE CHROMOSOME NUMBERS IN CAREX

Section and Subsection		X number
Sect.	Muehlenbergianae	29, ca. 29
Sect.	Acutae	
Subsect.	Rigidae	35
Subsect.	Vulgares	ca. 37, 42, 42, ca. 42
Subsect.	Caespitosae	40, 40
Sect.	Atratae	27, 28, ca. 37
Sect.	Pachystylae	24, 32
Sect.	Montanae	9, 15, 19
Sect.	Digitatae	ca. 23, 26
Sect.	Paniceae	16, 16
Sect.	Spiritostachyae	28, 33, 34, 34, 35, 37
Sect.	Physocarpae	
Subsect.	Lupulinae	26
Subsect.	Vesicariae	38, 41

It appears that species belonging to the same section have numbers of about the same magnitude. In the table groups of chromosome numbers are obtained,

corresponding to the taxonomic sections or subsections of the genus. In some cases the agreement is not as good as might be desired, but this probably results from the fact that some of the taxonomic groups investigated are more or less heterogeneous and not quite "natural." On the whole, however, the cytological-taxonomic interrelation is rather clear.

Since the above table was published I have brought together, in the botanic garden of Stockholm, a new collection of *Carex* species, which are now being investigated cytologically. As yet only very few new chromosome numbers have, however, been counted, but they agree well with the data in the above table. The new numbers are:

TABLE 2. DISTRIBUTION OF CHROMOSOME NUMBERS IN CAREX; RECENT COUNTS

Section and Subsection		X number
Sect.	Stenorhynchae	26, 34
Sect.	Acutae	
Subsect.	Cryptocarpae	42 (cf. Table 1.)
Sect.	Hymenochlaenae	
Subsect.	Capillares	27
Subsect.	Longirostres	29
Sect.	Physocarpae	
Subsect.	Vesicariae	about 40 (cf. Table 1.)

These new data on *Carex* thus once more confirm the correctness of the cyto-taxonomic interrelation which was outlined in my previous paper. I hope to be able to carry on the investigation, and further develop the extent of the interrelation.

In this case I have also considered it possible to draw phylogenetic conclusions, having arrived at the opinion that phylogenetic lines should be drawn from groups with low to those with high numbers. Against this conclusion critical doubts have been expressed by some authors. Thus, it has been argued that the chromosome numbers, hitherto known, are too few to permit of any safe conclusions. I would then point out here that there is as yet nothing that *contradicts* my opinion of the cyto-taxonomic interrelation in *Carex;* and, further, that the evidence from other plant genera speaks decidedly in favor of the correctness of this opinion. It has also been argued that, though the higher chromosome *numbers* within a genus probably are derived from the lower ones, this should not mean that *species-groups* with lower numbers are more primitive than nearly related groups with higher numbers. I can not agree with this last conclusion, and I am still convinced of the correctness of the opinions expressed in my previous paper, cited above. Once more I should like to emphasize that if a group of species, for other reasons, is supposed to have descended from another group (or *vice versa*), the group with higher chromosome numbers should be regarded as the younger one. A very thorough morphological and taxonomic knowledge of the species-groups is, however, a necessary supposition for such cyto-taxonomic conclusions. On chromosome numbers alone no phylogeny can be built up.

Of cytological investigations on critical species or species-groups I should like to draw attention to Clausen's well-known *Viola* studies and especially

to Jörgensen's recent investigations on Danish birches. One of Clausen's chief results is that the critical forms intermediate between *Viola tricolor* and *V. arvensis* are hybrids characterized by somewhat varying chromosome numbers and small irregularities in their reduction division. All the birches investigated by Jörgensen belong to the old Linnean species *Betula alba*. The opinion of the systematists on the limitations of the "species" and "varieties" of this critical group vary much. A recent Swedish author (Gunnarsson) thus speaks of no less than 5 different species with hybrids in all possible combinations. The 2 most extreme types of the group correspond to the species *B. verrucosa* and *B. pubescens*. Jörgensen finds that the former has 14 haploid chromosomes, the latter 28. One specimen, undoubtedly belonging to the hybrid *B. pubescens verrucosa* showed generally 21 gemini with occasional irregularities in meiosis. Several other specimens of doubtful position showed haploid chromosome numbers varying between 21 and 28. A complete analysis of the whole group can not be made with common taxonomic methods but may perhaps be carried out with the aid of cytological and genetic studies.

As a further instance of such cytological studies on critical species-groups, some studies on *Draba* (now in print), with which I have occupied myself during recent years, may be mentioned. I have made a cytological investigation of some critical Arctic and Scandinavian species which have been studied from the taxonomic side by Mrs. E. Ekman. I have especially tried to find out whether *D. magellanica* and *D. rupestris* are to be distinguished from each other also in chromosome number and whether each of them is a uniform species. The result has been that the forms of *D. rupestris* examined have the haploid chromosome number 24. The majority of the forms of *D. magellanica* examined have 32 chromosomes; whilst 2 forms of the latter species, 1 from Spitzbergen and 1 from Greenland, have 40; and 1, from Finland, 24 chromosomes. The chromosome numbers thus point to the existence of 4 different types of which 1 is *D. rupestris* (X = 24) and 1 typical (Scandinavian) *D. magellanica* (X = 32). The 40-chromosome type should perhaps be referred to a new (Arctic?) species, whilst the *D. magellanica* with 24 chromosomes is suspected of being a hybrid race. It is, however, at present impossible to say whether a decided connection between chromosome number and exterior characters may be found in this case. I have, besides, within *Draba* also found the numbers 8 and 16, the genus thus showing a typical picture of a multiple number-series (8, 16, 24, 32, 40).

THE NATURE AND FUNCTIONS OF PLASTIDS ESPECIALLY ELAIOPLASTS[1]

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Little progress has been made so far in working out the mechanism of division in plastids. The subject has taken on a new interest in recent years in connection with the numerous contentions that plastids arises from chondriosomes. The chondriosomes are also said to reproduce themselves by division, though these claims are still matters of contention. Because of difficulties of observation in the case of such minute bodies, no very definite statements are made as to the method of division of the chondriosomes.

We have, however, an increasing mass of observations as to the stages and methods of division in the plastids. If it be true that plastids arise by growth from chondriosomes, and that multiplication by division may take place at both stages, the range of our conceptions as to the nature of the division process is considerably widened.

The difficulties involved in all theories as to the mechanics of the complex changes involved in karyokinesis are apparently paralleled by the inadequacy of all theories yet proposed for the apparently simpler process of direct division; still, the undoubted fact that plastids as cell constituents arise by division has been one of the most effective stimuli to the development of pan-meristic viewpoints.

As a preliminary to a consideration of the data relating to the reproduction of plastids by division, we may note briefly their general cell relations and functions in cell metabolism. It was early recognized that plastids are essential to carbohydrate formation. Just how they function in this basic process is still, after decades of intensive study, in large degree a matter of dispute. In this connection we may note some observations which have been largely disregarded by students of photosynthesis. I refer to the work of Timberlake on the relation of the pyrenoid to starch formation in *Hydrodictyon*, and McAllister's account of parallel but quite distinct phenomena in *Anthoceros*. McAllister's observations on starch formation in *Anthoceros* are quite overlooked by Scherrer and Lorbeer in their accounts of the plastids and pyrenoids in this plant. In a word, Timberlake finds that the starch grains originate as segments successively cut off from the pyrenoid, which then grow in size and show the typical staining reactions of starch. McAllister finds that the pyrenoid of *Anthoceros* is an aggregate of spindle-shaped bodies, each of which is a starch grain initial. These bodies in

[1] Presented before the International Congress of Plant Sciences, Section of Cytology, Ithaca, New York, Aug. 19, 1926.

Anthoceros are at first in Flemming's triple stain erythrophile, but as they grow they become cyanophil just as do the pyrenoid segments in *Hydrodictyon*.

The obvious suggestion from these observations is that the first visible product in starch formation is the material of the pyrenoid, which it is generally agreed is protein, and that the starch grain initial arises secondarily, perhaps as a dissociation product, and then increases in size as it becomes the typical storage starch grain of the alga or liverwort cell.

The method of origin of the pyrenoid has been much studied and the general fact that it may arise both *de novo* and by division seems well established. As noted, the reproduction of plastids by division has in recent years become involved with that of the so-called chondriosomal origin of plastids. On this latter phase of the question I shall not attempt to judge the relative validity of the highly conflicting statements in the literature. Schürhoff has summarized the evidence on both sides very adequately.

Evidence has also been brought forward that aleurone grains and various other cell bodies also arise from chondriosomes. If this view is further confirmed it will make still more sharp the problem of the nature of the division process in mature plastids, since aleurone grains are not regarded as arising by division and their formation *in vitro* has been reported.

Even if it is assumed that the chondriosomes are endowed with the power of self-division, this by no means makes clear the nature of the mass transformation by which plastids constrict and pull apart into daughter halves.

The accounts of the division of plastids are as a rule fragmentary, and in many points inconsistent. Perhaps the fullest account of the division of such starch-containing bodies is that of Marquette for the cells of *Isoetes*. Marquette leaves it uncertain as to whether the bodies he studied are plastids or starch masses which divide prior to cell and nuclear division and thus distribute the accumulated carbohydrates to the young daughter cells. There can be no question, however, that these masses of starch grains, frequently enclosed in a conspicuous membrane, are the same structures as the plastids of *Anthoceros*, *Selaginella*, etc. That they divide in advance of the division of the nucleus and without any evidence of the presence of a centrosome or of karyokinetic spindles, is rightly emphasized by Marquette as a case of evident polar organization of the cells without the presence of a centrosome or astral system, showing that the transformation of the somewhat radial organization of the resting cell into the bipolar organization of the dividing cell does not require the presence of a dynamic center expressing itself in fields of force or systems of organic rays.

In the resting cell of *Isoetes* the plastid containing numerous starch grains lies beside the nucleus. Both may be lobed and may overlap each other, or they may be quite globular in outline and merely in contact, as is more common in *Anthoceros*. The plastid next elongates and constricts in its equatorial region, remaining in close contact with the nucleus and gliding over its surface. The nucleus may adhere to it and be pulled out along its surface of contact with the plastid. Ultimately the latter separates by breaking in its narrowed middle,

and the 2 halves round up on opposite sides of the nucleus, as it were, predetermining the poles of the future spindle and the plane of cell division.

This process of division of the starch body described by Marquette parallels that found by Scherrer and Lorbeer for the plastids of the gametophyte of *Anthoceros* and is in quite general agreement with the figures of von Mohl for the spore mother cells, though we have here the interesting situation that the plastids divide twice and take up symmetrically disposed positions with relation to each other and the nucleus before the latter begins its first division. In *Pellia* this situation is still more emphasized by the fact that the mother cell itself takes on a 4-lobed form before the nucleus begins to divide.

Von Mohl in 1839 figured correctly the various positions which the plastids could assume prophetically for the disposition of the future spores. He shows them arranged in a plane, in a tetrahedron, and criss-cross, which are the common approximations to least surface positions. No cogent suggestions are offered by any of these authors as to the nature of the process or the mechanical steps involved in the division of plastids.

Out of the very numerous accounts in the literature, it seems evident that chloroplasts divide, or are divided, in 2 ways: (1) by passive division, as in *Spirogyra*, *Mesocarpus*, etc.; (2) by more or less self-determined activity as in the higher plants, in *Isoetes*, *Anthoceros*, *Selaginella*, etc.

Futher, there are numerous reports indicating that this self-determined division may involve 2 more or less distinct types of change. It may occur by constriction without elongation, or sometimes with the formation in advance of a special hyaline cleavage zone.

Mikosch, in one of the early papers on plastid behavior, reports 2 types of division of the plastids in the aerial roots of *Hartwegia comosa:* (1) With preformed hyaline zone; (2) by direct constriction and elongation. He is confirmed by A. Meyer, who finds no starch in *Hartwegia* but oil droplets in the plastid.

Haberlandt reports that in *Selaginella* the chloroplasts show all grades from division with the appearance of a colorless zone to ordinary constriction. These 2 methods of division, with and without the preliminary formation of a hyaline cleavage zone, run parallel with the conditions in the cleavage of the aethalium of *Fuligo* into spores. At certain stages the formation of cleavage furrows is preceded by the appearance of well marked cleavage zones which mark off in advance the spore bodies which are to be formed. In other cases the constriction furrows cut into quite undifferentiated cytoplasm and their orientation is apparently quite autonomous. In neither case is there elongation of the dividing mass in *Fuligo*.

The division figures of the nuclei in *Empusa*, as shown by Olive, as the daughter chromatin masses separate within the nuclear membrane, are strikingly like certain stages in plastid division with the formation of a cleavage zone. Of course no nucleolo-centrosome is present in the plastid, and this parallelism suggests no other similarity than that of some underlying property of such colloidal masses.

Von Mohl (1839), as noted, observed in the plastid of *Anthoceros* division without elongation and with the formation of fibrils stretched between the daughter plastids as the latter drew apart. These figures suggest the existence of systems of fibers between the daughter plastids like those between daughter nuclei in cytokinesis with cell plate formation.

Strasburger describes the plastids as dividing without separation. They then separate and fibers appear between them. The nucleus divides twice and simultaneous cell division with cell plates follows. The fibers of von Mohl's figures are a part of the phragmoplast system, as Strasburger finds. Němec describes the division in *Anthoceros* as by constriction with elongation. Lorbeer gives figures of division of the chromosomes in *Anthoceros* like Marquette's for *Isoetes*.

Kiyohara in a recent paper reports the finding in *Hydrilla verticillata* of a very interesting type for the study of the division of the chloroplasts. In this plant they can be studied in the living cells and are carried along in the rotating stream of cytoplasm. They show a stroma of protein and grana of chlorophyll, and in addition a highly refractive nucleole-like body whose significance Kiyohara does not discuss.

In the young leaves of *Hydrilla*, 3 zones can be distinguished on the basis of the appearance of the chloroplasts. In the median region the plastids are constricted, while at the base and apex of the leaf they are disk-shaped and entire. There is a daily periodicity in the division of the plastids. At 5 A.M. a cleavage zone is first to be distinguished. Through the day the process of constriction goes on slowly. From 8 to 11 P.M. the process is speeded up and is completed by 1 A.M.

The stages of division are: (1) Elongation of the plastid and doubling of the refractive body. (2) A cleavage zone next appears, at first colorless, then yellowish, then colorless. (3) Constriction. (4) The halves pull apart with long strands between them. They may separate suddenly, or remain connected for an hour or so.

Kiyohara's contribution, based on what is evidently very favorable material, seems to favor the possibility that division is initiated by the appearance of a special zone, comparable perhaps to a cell plate or phragmoplast. On the basis of Strasburger's view as to the makeup of the cell plate this zone may be made up of granules which divide and thus determine the cleavage of the plastid. Such a view does not, however, any more than in the case of the chromosomes, complete the transformation of the monocentric into a dicentric system. Constriction and the pulling apart of the daughter halves may be a protracted process involving the activity of forces not at all concerned in the division of the possible granules in the cleavage zone.

With the appearance of Kiyohara's work we may say, perhaps, that the accounts of the methods of division of plastids are about equally distributed between those reporting the formation of an initial cleavage zone implying a possibly complex internal mechanical organization of the plastid, and those

reporting direct division by a cleavage furrow, implying nothing more than a simple mass constriction.

My own studies of the process in *Anthoceros* have so far failed to show any evidence of the formation of a cleavage zone in advance of, or simultaneous with, the equatorial constriction and pulling apart of the plastid halves. My results are in agreement with those of Lorbeer and Scherrer, but one of McAllister's figures not specially discussed by him might suggest the existence of a cleavage zone.

With the rapidly increasing evidence that the elaioplasts are widespread and in many cases highly specialized cell structures, the problem as to their relation to the chloro- and leucoplasts becomes increasingly important. As I have earlier pointed out, in the elaioplasts we may have transition forms between undifferentiated ergastic aggregates in the cell and those which have come to occupy such specialized loci for certain functions as to maintain themselves from one cell generation to another by division. Certain types of elaioplasts are just as definitely organized, so far as visible structure and apparent functions are concerned, as are the chloroplasts. There is as yet, however, no adequate evidence that even the most specialized elaioplasts divide autonomously. Schürhoff says they divide passively.

Beer holds that certain very indefinitely localized, oil-containing masses in the cells of some of the Compositae are aggregates of degenerating plastids. These masses found by Beer are quite different in their visible structure from the elaioplasts of vanilla, *Ornithogalum*, and at least many of the host of new forms described by Politis and others. These bodies described by Beer may well represent, as he claims, merely degeneration products of the plastids in which oil appears as a dissociation product of protein.

Among the cases now described we may distinguish at least three types of conditions in which fatty materials are found in plant cells; and we can perhaps arrange these in an ascending series as to their specificity in localization and degree of organization.

(1) The first type is that in which oil occurs as finely divided droplets distributed more or less irregularly in the cytoplasm, as found in the storage tissues of seeds and in many other plant tissues. Fatty materials which are destined to be again taken up into the cell metabolism are most commonly found in this emulsified condition in the cytoplasm.

(2) Oil bodies. Larger masses or aggregates of oil drops but without a stroma are found by Stein singly in epidermal cells of *Ludwigia* and in many other plants. Küster finds them in *Sambucus* and *Saxifraga*, and says that there is no membrane or stroma in these forms. They constitute a stage, perhaps, between the finely emulsified droplet type and the elaioplasts with more differentiated organization, and indicate clearly the tendency to the definite localization of the oil-forming process in a certain part of the cell.

(3) Elaioplasts with a well marked stroma in which oil drops are imbedded. Raciborski was able to differentiate the stroma sharply by appropriate staining methods, and he claims that it shows specific protein reactions. Wakker thought

that the elaioplasts in vanilla were metamorphosed chloroplasts, but the numerous studies which have appeared since his day have not brought convincing evidence for this view.

Küster has shown that the stroma can be recognized in cells of *Ornithogalum* before the oil appears in it. I have studied the young leaves of *Ornithogalum*, and can confirm Küster's statement that the stroma in early stages shows no oil, or very little oil. Later on the oil becomes abundant and in the ordinary processes of imbedding and sectioning it may be extruded on the surface of the stroma. Whether the oil is more or less abundant the stroma is always definitely differentiated from it.

The distribution of the elaioplasts in the tissues of *Ornithogalum* in which they occur is very uniform. Typically there is a single elaioplast in each cell in *O. umbellatum*. Zimmermann finds clustered oil bodies in certain other species of *Ornithogalum*. The uniform distribution of the elaioplasts suggests that they multiply by division but, as noted above, no convincing evidence on this point has yet appeared.

The idea that such bodies as elaioplasts are in some fashion agents rather than merely loci for specific reactions in the cells, is still almost universally accepted. This conception is embodied in the doctrine of the so-called individuality of cell organs. It seems to me, however, that we shall do well as a working hypothesis to assume that whatever a given organ does or seems to do can only be regarded as an expression of the metabolism of that organ or of adjacent organs acting upon it. It is an agent only in the sense that it is a locus for a specific activity. The assumption that minute particles assumed to be endowed with various life properties are the ultimate constituents of the cell has not cleared up the problems involved in the mechanics of either cell or plastid division.

MEIOSIS IN THE POLLEN MOTHER CELLS OF THE OENOTHERAS, AND ITS PROBABLE BEARING UPON CERTAIN GENETICAL PROBLEMS[1]

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For the past 7 years, I have been studying meiosis in the pollen mother cells of the evening primroses. Up to the present time, a total of 5 species, 11 varieties or mutants, and 2 sets of reciprocal F_1 hybrids have been examined, and in addition I have under cultivation this summer 6 species, 3 mutant races, and 8 F_1 hybrids.

In the time allotted, it will not be possible to discuss the meiotic process in detail, but I shall confine my attention to certain specific phases, particularly to the stages of diakinesis and the heterotypic metaphase. The earlier stages may be passed over without comment, except perhaps to say that all the evidence seems to favor a telosynaptic interpretation, rather than a parasynaptic one. With this brief statement, I shall pass immediately to a consideration of diakinesis.

The most striking phenomena displayed by the Oenotheras are associated with the end of prophase, generally called "diakinesis," and with the heterotypic metaphase. In most plants, diakinesis is a period during which the various pairs of homologous chromosomes separate from one another and stand apart in the nucleus, each pair entirely independent of the others. In the evening primroses, however, exceptions to this behavior are so frequently met with that they seem to be more the rule than the exception. Ever since the Oenotheras were first investigated cytologically, it has been recognized that the uniform pairing of univalent chromosomes at this stage is to a greater or less degree suppressed or lacking. At first it seemed that this fact could be interpreted only as due to a disturbance of some sort, resulting in a breakdown in orderly procedure, such as might conceivably accompany a period of mutative activity, or a hybrid constitution. But my studies have shown that the situation is far from being a chaotic one. The deviations found in *Oenothera* from the ordinary rule of behavior in diakinesis themselves conform to rule.

The essential features of diakinesis in the Oenotheras may be summed up as follows:

(1) The number of chromosomes which fail to pair in a nucleus is constant, not only for a given plant, but apparently for each entire race of plants in which

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failure to pair occurs. Thus, in *O. Lamarckiana*, 12 chromosomes fail to pair in all of the plants that I have examined.

(2) The number of unpaired chromosomes varies throughout the genus from none in some races to the whole diploid number of 14 in others. At least some of the chromosomes fail to pair in about two-thirds of the races that I have studied. It is worthy of note, however, in view of the supposed general lack of pairing in the genus, that there are strains of evening primrose in which complete pairing is normally found (see Cleland, 1923, 1925).

(3) All unpaired chromosomes in a nucleus become associated into 1 group, and are found to be united end to end in "diakinesis," to form a closed circle. The only exceptions to this so far observed are in the cases of *O. biennis* and *O. biennis sulfurea*, where there are 2 such circles, one composed of 8 chromosomes and the other of 6. Up to the present time, I have discovered no undoubted cases of departure from the arrangement characteristic of a given species at this period. Thousands of nuclei have been studied, and in practically every instance in which all chromosomes have been visible, the arrangement has been found to be that characteristic of the species under investigation. The only form in which I have found any amount of variation at this time is *O. oblonga*, a 15-chromosome mutant from *O. Lamarckiana*, in which there is a certain range of variation in the number of chromosomes that may be paired or unpaired. Even in this form, however, all unpaired chromosomes, no matter what their number, become associated into one group.

(4) Paired chromosomes are generally observed to be in the form of small rings.

(5) Detailed study indicates that the rings bear in their positions a definite relation to each other and to the circle, if such be present. When the second contraction knot unfolds, they are found to be linked to one another, and to the circle, in various ways, and the arrangement seems to be uniform for each species. I have worked this out with precision in only a few forms, but have seen enough of all species examined to make me feel certain that the chromosomes are arranged in a very definite way in each of the species. Thus, in *O. franciscana* there are 3 rings linked to the circle of 4, and to 2 of these another ring is linked. In *O. rubricalyx*, with its circle of 8, the 3 rings are all linked to the circle. In *O. Lamarckiana*, the single ring is linked to the circle. The chromosomes, whether paired or unpaired, therefore, are arranged according to a definite scheme, and present the same configuration in all cells. Nothing could be more regular and more orderly than the phenomena displayed during diakinesis in the Oenotheras, and yet, how different in many instances from the type of diakiesis normally observed in other species. Contrast *O. muricata*, for example, having 14 unpaired chromosomes, with the typical diakinesis picture, such as is shown by *O. blandina*. The difference is great, but in the former, just as much as in the latter case, regularity and strict conformity to rule are to be seen. We can therefore no longer think of the phenomena displayed by the Oenotheras during diakinesis as disturbances, as enforced departures from an orderly course of events. In these plants, the laws are different, but none the less fixed. The

result is a type of behavior that is practically without parallel among plants and animals as yet studied.

When we pass to the next stage, the heterotypic metaphase, even more singular phenomena greet us. Although paired chromosomes follow the same history as do the bivalents in other genera, the unpaired chromosomes, comprising the circles, behave in a very peculiar manner. Briefly, the procedure is as follows: The circles usually remain intact throughout metaphase. Occasionally they break open at one point and form a chain rather than a circle, but in any case, the group remains together, and does not break up normally until the chromosomes are separated to the poles in anaphase. For a time after the disappearance of the nuclear membrane, they lie apparently without orientation in the center of the cell. Gradually, however, the chromosomes in the still intact circle become arranged with reference to the poles according to a fixed scheme. Sheaves of spindle fibers from one pole or the other become fastened to the middle of each chromosome, in such a way that adjacent chromosomes become drawn toward opposite poles. This results presently in a lining up of the chromosomes of the circle across the equatorial region, and as the pull exerted by the fibers (or the attraction from the poles which is expressed in the fibers) becomes stronger, each chromosome, being held back by its attachment to adjacent chromosomes which are being pulled in the opposite direction, becomes somewhat V-shaped, and the circle as a whole assumes a regular zigzag appearance which is as striking as it is unique. In most species possessing circles, the appearance in mid-metaphase as seen from the side is one of great regularity, providing the fixation has been adequate. Seven chromosomes point toward one pole, and between and attached to each 2 of these is a chromosome pointing toward the other pole. Those being pulled to a given pole tend to lie, in most species, at approximately the same level, and as they become separated more and more from those destined for the other pole, an open space develops between the two sets.

We find, therefore, that the process in metaphase is as orderly as it was in late prophase, and as unique. So far as I am aware, the presence of an intact circle in metaphase, and the regular distribution of alternate chromosomes to the same pole in anaphase, have not been observed in any other group of organisms. As a result of this behavior, 7 chromosomes are regularly distributed to each pole.

While exceptions to the specific arrangement of chromosomes in diakinesis never, or almost never occur, the same can not be said in regard to the zigzag arrangement in metaphase. In fact, such irregularities commonly do occur in the larger circles in proportions varying from approximately 10 to 20 per cent in the different species. Such irregularities rarely if ever cause a complete upset in the zigzag arrangement. Even when they are present, most of the chromosomes still maintain their normal position, visible irregularities involving at most only 3 or 4 chromosomes.

The stages that succeed the heterotypic metaphase are not significantly different in the Oenotheras from the corresponding stages in other plants, and consequently we shall pass them by at this time. It now remains to discuss the

possible significance of the facts just presented. And first, I wish to discuss briefly 2 questions relating to the nature of the chromosomes in the Oenotheras. The first concerns the relative positions of the individual chromosomes within the nucleus, and the second, the reasons for the lack of pairing of the homologous chromosomes found in the circles.

In regard to the first of these, it seems probable that the chromosomes comprising the circles have definitely fixed positions within the circles, such that homologous chromosomes are normally separated to opposite poles, and the same chromosomes pass together to the poles in all cells. Direct proof of this supposition can not be obtained, because of the uniformity in size and shape of the chromosomes. The result, however, would be disastrous, were the chromosomes placed merely according to chance in the circles. We have seen that the circles remain intact throughout metaphase, and adjacent chromosomes pass regularly into different daughter nuclei. If the chromosomes were not definitely arranged with reference to this mode of distribution, an impossibly large amount of non-disjunction would be the result. For instance, in a circle of 12 chromosomes, the univalents may be arranged in any one of $\lfloor 11$ ways, and they may be so arranged that all homologous chromosomes will be separated and go to opposite poles in $\lfloor 6 \lfloor 5$ ways. The ratio of the latter to the former is 1:462; in other words, only one cell in 463 would, on an average, be entirely free from non-disjunction if the order of arrangement of the univalent chromosomes in a circle of 12 were entirely a matter of chance in the various cells. In a circle of 14 chromosomes, the ratio would be 1:1716,—there would be but one chance in 1717, on the average, of entire freedom from non-disjunction. It is manifestly absurd to suppose that *O. Lamarckiana*, with its circle of 12, and *O. muricata*, with its circle of 14, experience any such overwhelming amount of non-disjunction. The chromosomes must be so placed within the circles that the mechanism just described separates as a rule the members of the various sets of homologues from each other and carries them to opposite poles. The simplest arrangement which would bring about this result would be that in which the homologous chromosomes were placed side by side in the circle, so that, being adjacent, they would surely be separated, under normal conditions.

It is furthermore probable, not only that homologous chromosomes are so placed as to be separated in anaphase, but that the various pairs of homologues are also definitely situated with reference to one another. This is quite probable in view of the fact that in species in which the circle is associated with one or more sets of paired chromosomes, these are all linked together in diakinesis after a very definite fashion, characteristic of each species. This would seem to indicate that in the earlier stages of meiosis the various chromosomes are arranged in a certain fixed way in each species, so that when they finally emerge from second contraction, they are found to be linked in the same way in all cells. If this process results in the chromosomes outside the circles having fixed positions with reference to one another and to the circle, there is strong reason to suppose that the same holds true in the case of those within the circles also.

A still further point has to do with the position of the individual univalent

chromosomes within the pairs in the circles. If the chromosome pairs are as uniformly placed as it seems likely they are, and if homologous chromosomes are so placed that they may normally be disjoined, it seems probable that the final step has also been taken, namely, that the position of each member of a pair of homologues is definitely fixed with reference to the circle as a whole. Thus, the sets of homologous chromosomes A and A′, B and B′, C and C′ would be placed in a circle in the order AA′ BB′ CC′, and never normally in the order A′ ABB′ CC′. This last point is an important one. If it is a matter of chance whether the arrangement is AA′ BB′ CC′ or A′ ABB′ CC′, the genetical result of meiosis in Oenothera ought to differ in no way from the results in other plants. If, on the other hand, their positions are fixed, and AA′ BB′ CC′ is the normal arrangement in all cells, then it will mean that, with adjacent chromosomes going to opposite poles, A will always go to the same cell with B and C, never with B′ or C′, irregularities excepted; and this is in line with the genetical situation in the genus. We shall assume, therefore, that univalent chromosomes are placed in definitely fixed positions within the circles with reference to all other univalent chromosomes, in such a manner that homologous chromosomes are separated to opposite poles in the reduction division, and the same chromosomes pass together to the poles in every cell.

The second question has to do with the reason for the presence of circles in some species and their absence in others. More than one possibility presents itself in this connection. It might be assumed that the failure to pair on the part of certain chromosomes is due to their being to a relatively high degree heterozygous; whereas ability to pair is a mark of a relatively homozygous condition. In other words, an increasing degree of genetical dissimilarity between homologous chromosomes entails an increased amount of incompatibility between them, until the point is reached beyond which the homologous chromosomes are no longer strongly attracted to each other. If this suggestion be correct, the major portion of the heterozygous genes are to be found in the chromosomes comprising the circles, and comparatively few, if any, are in the paired chromosomes.

Opposed to this conception is the possibility that the presence of circles is an inherited characteristic, their presence and size being dependent upon the functioning of certain genes, just as in the case of any external characteristic. The circles would not then bear any necessary relation to the position of heterozygous genes. It ought to be possible to determine whether this is the situation or not by a study of the inheritance of circles in crosses. Data of this nature, however, are as yet too meagre to be of value. I have examined only 2 sets of reciprocal F_1s, one between *O. franciscana* and *O. grandiflora*, and the other between *O. grandiflora* and a form called by Shull "mut. *sulfurea*." The strains of *O. franciscana* and *O. grandiflora* used in the first case both lacked circles, but both reciprocals had a circle of 4. Mut. *sulfurea* has a circle of 6, and in both reciprocals of the cross between it and *O. grandiflora*, some plants had a circle of 6, and some no circles. The similarity between the chromosome configurations in the F_1s and in the parents, especially in the latter case, is interesting, but

until more data of a similar nature can be compiled, these facts can not be considered particularly significant.

Of the 2 alternatives, the former seems at present to have the most in its favor, namely, that the failure to pair, and consequent association into circles, are due to incompatibility between the homologous chromosomes, dependent upon a high degree of heterozygosis. In the first place, it is known that in a good many hybrids homologous chromosomes fail to pair, apparently because of a lack of affinity due to divergence in constitution. It may be that in the Oenotheras a failure to pair likewise indicates divergence in the genetical constitution of the homologous chromosomes. In the second place, it is a very striking fact that, on the one hand, all of the permanently heterozygous species of *Oenothera* which have been examined display large circles, whereas, on the the other hand, all of those known to be homozygous, or suspected of being such, have shown an absence of circles, or at most, very small ones. Species which might be considered in an intermediate condition, such as the so-called half-mutants of de Vries, are also intermediate in having medium-sized circles. For instance, *O. Lamarckiana*, *O. biennis*, and *O. muricata*, which are permanently heterozygous species, all have large circles. An interesting culture grown last year by Professor Shull, which was heterozygous for 7 of the characters which he has studied—in fact, the most heterozygous form he has obtained so far—shows in one plant which I tested a circle of 10, and in another a circle of 12 chromosomes. On the other hand, *O. deserens*, *O. blandina* and *O. latifrons*, which de Vries considers homozygous races, all lack circles. The strain of *O. grandiflora* isolated by Davis has no circles, and *O. franciscana*, which I studied in 1919, has a circle of but 4 chromosomes, while the continuation of this strain grown by Shull lacks circles altogether. These 2 species are considered by Davis at least to approach the condition of pure species. *O. rubrinervis* and *O. erythrina* are derivatives of *O. Lamarckiana*, and are styled half-mutants by de Vries. They are intermediate between the permanently heterozygous *O. Lamarckiana* and the permanently homozygous *O. deserens* and *O. decipiens* respectively. Both of these plants have a circle of 6 chromosomes, and *O. rubricalyx*, which is intermediate between *O. Lamarckiana* and *O. latifrons*, has a circle of 8. Thus it would seem that, so far as my work has gone, a direct relation can be traced between the presence of circles of chromosomes and the degree of heterozygosis displayed.

A third factor also enters into this correlation, and that is the presence or absence of lethals. All of the permanently heterozygous species possess balanced lethals, and the intermediate forms have an unbalanced condition with 1 of the 2 complexes free of lethals; while the homozygous races are alethal. We have then a correlation between, on the one hand, a high degree of heterozygosis, the presence of balanced lethals, and large chromosome circles; and on the other, a homozygous condition, no lethals, and the absence of circles of chromosomes, with the correlation holding true in intermediate forms as well, at least so far as my studies have taken me.

If the assumption be true that unpaired chromosomes are relatively heterozygous, and paired ones are relatively homozygous, it would seem at first sight

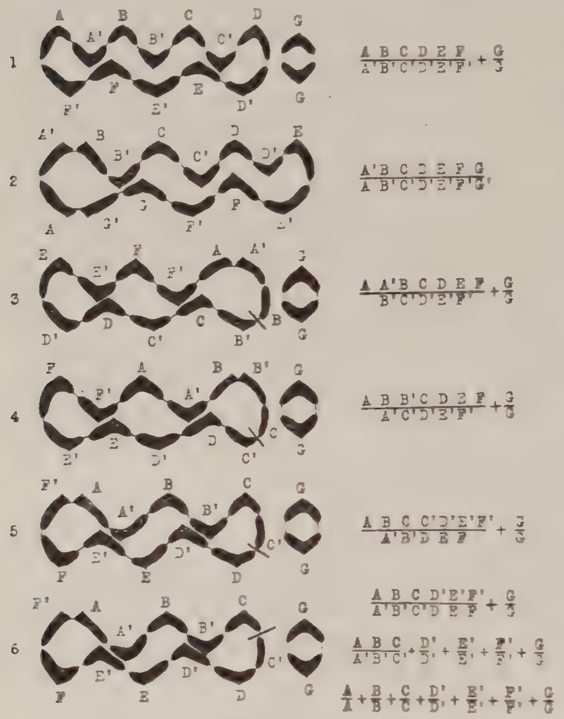
that interspecific hybrids would show rather large circles. It was with this thought in mind that I grew and examined the reciprocal F_1s between *O. franciscana* and *O. grandiflora*. Both species lack circles. I expected to find large circles in the F_1s, since it seemed that there would naturally be a considerable amount of heterozygosis. But both F_1s showed only a circle of 4 chromosomes. The reason for this, however, may perhaps be sought in the light of some recent work of Renner ('25). From his analysis of the complexes of various species, the striking fact is disclosed that in a number of cases complexes belonging to different species are possibly more nearly akin than the 2 associated complexes making up the species themselves. It is possible, then that the F_1s between *O. grandiflora* and *O. franciscana* are actually rather closely allied. However, no conclusions can be drawn regarding the evidence to be obtained from a study of hybrids until more of them have been examined.

To sum up, it seems reasonable to adopt the following 2 assumptions as working hypotheses: (1) The univalent chromosomes in the circles have definitely fixed positions, so that homologous chromosomes are normally separated in anaphase, and the same chromosomes go together to the poles in all cells, hence these become consistently associated into the same 2 complexes within the daughter nuclei. (2) Most of the heterozygous genes are to be found in the chromosomes comprising the circles. With the aid of these assumptions, it becomes possible to offer an explanation based upon chromosome behavior in meiosis for some of the genetical peculiarities of the Oenotheras.

One of the striking characteristics of the Oenotheras is the existence of very extensive complexes of linked genes. In fact, in the various wild strains now under cultivation, all or almost all of the known genes belong apparently to a single linkage system. It is as though practically all of the genes were situated in a single chromosome pair, or as though the Oenotheras had but one pair of chromosomes, instead of 7. These plants are then homozygous or heterozygous, not for limited groups of genes, but for the whole complex. Using the terminology of Renner, they are "complex-homozygotes" or "complex-heterozygotes," depending upon whether they are pure or impure for the single linkage group. Plants with only one linkage group can, of course, form normally but 2 kinds of gametes. Moreover, it naturally follows from such extensive linkage that the germ cells formed by such a plant will be the same in kind as those by the union of which it was originally formed. Paternal and maternal complexes are therefore passed on intact to the next generation. Cross-overs excepted, there is apparently no shuffling, no independent assortment of genes in the reduction divisions.

Our first problem, then, is to account for the fact that with 7 sets of homologous chromosomes, the various species have but one linkage group. Shull, who has found that most of the factors which he has studied belong to one linkage system, has been inclined to assign all of these factors to a single chromosome, and to explain the linkage between them as due to this fact. He suggests that most of the factors which distinguish the various species reside in a single chromosome, and that the other chromosomes are relatively free from such

factors. Renner, on the other hand, believes that the linked factors are scattered throughout the various chromosomes and that there is some mechanism which effects the linking of separate chromosomes into a larger physiological unit. The latter view comes closer to being in line with the cytological facts. Shull's theory takes for granted a normal chromosome behavior. Renner's calls for a peculiar mechanism, bringing about linkage of the genes lying in different chromosomes. This mechanism is to be found, I believe, in the peculiar way in which the chromosomes are arranged and distributed in the heterotypic division.



Figs. 1.—6.

In order to demonstrate this fact, let us take for illustration a form having a circle of 12 chromosomes and one pair, such as *O. Lamarckiana* (Fig. 1). The homologous chromosomes in the circle, which are relatively heterozygous, we will designate by letters AA′ BB′, etc., and will assume that they are placed within the circle in the following order,—AA′BB′ CC′DD′EE′FF′. Since as a rule adjacent chromosomes go to opposite poles and alternate chromosomes to the same pole, chromosomes ABCDEF should go to the same pole in all normally behaving cells, and A′–F′ to the other. It is as though chromosomes ABCDEF were organically united, and were but chromomeres belonging to one large univalent chromosome. Genes in chromosomes A and F are as much linked genetically as though they were in the same chromosome. The circle, therefore, constitutes a single large linkage group. Furthermore, since the chromosomes in the circle are probably heterozygous to a large degree, the complexes of genes in A-F and in A′-F′ are no doubt more or less dissimilar, so that the daughter nuclei and resultant reproductive cells are probably unlike in many respects.

The 2 paired chromosomes, which are not a part of the circle, are assumed to be relatively homozygous, and since they have few if any heterozygous allelomorphs, they will not have any marked influence on the number of gametes formed. To all intents and purposes, therefore, we have but 2 complexes, and 2 types of gamete formed.

If we take for illustration a plant with a smaller circle, and a larger number of paired chromosomes, the same will probably hold true. The circle is a single large linkage group, and the complexes formed by the disjunction of adjacent chromosomes will be somewhat dissimilar, owing to the heterozygous character of the chromosomes involved, but probably not as dissimilar as in species possessing larger circles. Paired chromosomes, though more numerous, will have scarcely any effect upon the number of gametes formed, being relatively or even entirely free from heterozygous allelomorphs. These plants will also have, then, but 2 types of gamete. Nevertheless, there is the possibility that as many additional linkage groups may be discovered as there are sets of paired chromosomes in such a plant. A single recognizable pair of heterozygous factors in one of the paired chromosomes will serve as the marker of a new linkage group. The presence of paired chromosomes probably accounts for the fact that in addition to the one large group, Shull has discovered 2 much smaller groups.

Some species, as we have seen, have no circles at all. These, from their genetical behavior, are considered homozygous species, and this is in keeping with the supposition that paired chromosomes are relatively free from heterozygous factors.

All things considered, therefore, it would seem that chromosome behavior in meiosis in the Oenotheras is probably at the basis of the extensive linkage, and of the presence of complexes which involve most if not all of the hereditary factors, and which are passed on intact from generation to generation.

A second problem closely related to the one we have just discussed concerns the presence of crossing-over in the Oenotheras. Is there any cytological basis in these telosynaptic plants for the phenomenon in question? Crossing-over, as ordinarily thought of, is a process that is supposed to occur within a single chromosome pair. We have suggested, however, that in most Oenotheras genes that are in separate chromosomes may be as completely linked as though they were in the same chromosome, and it now remains to be shown that crossing-over can also occur between such genes as well as between those residing in the same chromosome. For a mechanism capable of yielding this type of crossing-over, we must look to the various types of irregularity which sometimes occur in the zigzag arrangement in the heterotypic metaphase, and which result in abnormal distribution of the chromosomes to the poles. I shall give an example or two which will illustrate the probable importance of such irregularities.

One type that I have observed is indicated in Figure 2. Two adjacent chromosomes, instead of going to opposite poles, have been caught by fibers leading to the same pole, and the next pair are destined to pass together to the opposite pole, instead of separating. The other chromosomes are arranged in the regular zigzag fashion. Let us suppose that the irregularity has occurred in such a way that the adjacent chromosomes passing together to the poles are members of different sets of homologues, as indicated in the figure. The complexes that will result in this particular case will be $\frac{A' \; B \; C \; D \; E \; F \; G}{AB'C'D'E'F'G'}$. A and A′ have exchanged positions, and are in

the wrong complex. If now the upper complex functions in the formation of a hybrid zygote, the resultant F′ individual will probably show crossing over in the case of one or more characters, owing to the fact that it possesses A′ instead of A. What has happened is comparable to what takes place when sections of individual chromosomes are exchanged, only on a larger scale. Such interchromosomal crossing over no doubt involves a large number of genes, but as yet we know about only a few of them and it is not surprising that observed crossovers seem only to involve a single factor. This illustration will serve to show the possibility of the occurrence of crossovers between genes in non-homologous chromosomes, due to irregular arrangement and distribution of chromosomes in the heterotypic metaphase and anaphase. It should be borne in mind that this process does not exclude the possibility of the occurrence of crossing over of the ordinary intrachromosomal type, which I believe may also occur in these plants.

We come now to a third most interesting characteristic of the evening primroses, namely, their ability to give rise to mutant races, or mutations, using the term in its de Vriesian sense. Does chromosome behavior shed any light upon the origin of aberrant forms?

The distinctive feature of *Oenothera* mutations is the fact that in many cases they involve the simultaneous appearance of a whole group of mutated factors, in contrast to *Drosophila* and other organisms, in which most mutations concern but a single gene or at most a very small group. So characteristic of the Oenotheras is this type that de Vries has distinguished the mutations falling in this category as "typical" mutations, as composed to the *Drosophila*-like or "atypical" ones.

The reason for the sudden appearance of a number of mutated factors constituting together a de Vriesian mutation, is to be found, I believe, in the way in which the chromosomes are linked together and distributed to the poles. We have seen that in the various species the genes are all or almost all united into a single linkage group. In most species, each of the 2 complexes has a lethal which either kills it in sperm or egg, or makes it incapable of existing in the homozygous condition. With such a balanced-lethal situation, the only plants which can survive are those formed by the union of unlike complexes. In consequence of this permanently heterozygous condition, recessive genes are permanently hidden within the complexes, unless released through the agency of crossing-over, and at the same time, although permanently hidden, they can never be lost from the species. If, then, we suppose that from time to time mutations occur within the chromosomes, those which are recessive will gradually accumulate in the various chromosomes of the complexes, since they are linked with and preserved by the lethals, and their existence will never be suspected so long as crossing-over does not involve them. When crossing-over of the ordinary type occurs, involving only a portion of a chromosome, one or more of these mutated genes may be freed from the lethal and stand revealed, but when through the irregular arrangement and distribution of chromosomes one or more whole chromosomes become freed from the lethal in the process that I have termed interchromosomal crossing-over, more of these hidden mutated genes

are likely to be affected, and instead of but one making its appearance, a whole group will suddenly come to light, and the resultant individual, differing in so many respects from the parent form, might easily fall into the class of de Vriesian mutations.

I shall confine myself to giving one or two illustrations to show how this may take place. A great many of the so-called typical or de Vriesian mutations are trisomic in the case of one or more of the chromosomes. De Vries has divided these into 7 groups, each of which he assigns to a separate one of the 7 pairs of chromosomes—that is, all of the mutable factors involved in the production of any of these mutations are believed to be situated in a single chromosome, and thus to be closely linked. It seems probable that the chromosome which is doubled in each case is a member of the pair containing the factors involved in the appearance of the mutation.

It is then of interest to find that certain types of irregularity in the arrangement and distribution of chromosomes may give, if my working hypotheses are correct, the types of non-disjunction which seem to accompany, or perhaps occasion, the mutations under consideration. For instance, in *O. Lamarckiana*, when no irregularity occurs in the zigzag arrangement, the complexes formed should be $\frac{\text{A B C D E F}}{\text{A}'\text{B}'\text{C}'\text{D}'\text{E}'\text{F}'}+\frac{\text{G}}{\text{G}}$. But let us suppose that an irregularity occurs of the type illustrated in Figure 3, a kind which I have often seen. If it occurs at a certain point in the circle, as shown in the figure, the resulting complexes will be $\frac{\text{AA}'\text{BCDEF}}{\text{B}'\text{C}'\text{D}'\text{E}'\text{F}'}+\frac{\text{G}}{\text{G}}$. Non-disjunction has occurred in the case of chromosomes A and A′. If now an egg with this complex unites with a sperm containing the normal A′−F′+G, the resulting 15-chromosome plant will have an extra A′ chromosome. If the irregularity occurs somewhere else, as for instance in Figure 4, non-disjunction will involve some other chromosome. Thus it will depend on where the irregularity occurs in the circle as to what chromosome will be affected, and there is theoretically as much possibility of one chromosome being duplicated as another, leaving lethals out of consideration.

But there are only 6 sets of homologues in the circle, and de Vries has demonstrated 7 main types of 15-chromosome mutations. One of the 7, *pulla*, appears very rarely, and is considered by de Vries to belong to the central chromosome because it deviates only very slightly from *O. Lamarckiana*. It is possible that this form occurs through the non-disjunction of the paired set of homologues not included in the circle. Non-disjunction probably occurs very rarely in this pair, since it is independent of the irregularities taking place in the circle. Furthermore, the very fact that it is not incorporated within the circle probably indicates that it is relatively homozygous, hence relatively free from mutated genes. Consequently it is to be expected that duplication in this case would result in very little deviation from the normal, and that the 15-

chromosome plant would be almost identical with *O. Lamarckiana* taxonomically.

The type of irregularity just presented is by no means the only one which can give rise to complexes with 8 chromosomes. Other types that I have studied may also cause an unequal distribution to the poles. As a result of some of these, as for instance in Figure 5, not 1, but several chromosomes may become transferred from the right to the wrong complex. It is not likely, however, that any of these will result in the type of mutation under consideration, for these mutations, being dimorphic in nature, give rise, when selfed, to a large class of *O. Lamarckiana* plants, whereas the complexes formed by such irregularities could not possibly do so.

Another interesting group of mutations involves the so-called half-mutants and the homozygous races derived from them. Half-mutants are so called because of their intermediate position between the parent species and these homozygous strains. When selfed, they produce the following result: sterile seeds, 25 per cent; half-mutant repeated, 50 per cent; new form, which breeds true, 25 per cent. Examples of half-mutants are *rubrinervis*, *rubricalyx*, and *erythrina*. These plants seem, among other changes, to have lost the lethal factor from the *laeta* complex, while retaining it in the *velutina*. Consequently, upon being selfed, the modified *laeta* complex is capable of combining with itself, thus yielding a homozygous form, while the other, because of its lethal, can still exist only in a heterozygous state. *Rubrinervis*, selfed, yields in addition to itself the homozygous race *deserens*. *Rubricalyx* gives *latifrons*, and *erythrina* produces *decipiens*. *Deserens*, *latifrons*, and *decipiens* are entirely homozygous races. Their paternal and maternal gene complexes are identical. They have no lethals, breed entirely true, and give rise to no mutants. They may be said to represent what is generally considered an anomaly in plants; namely, an entirely pure species. Another homozygous race, *blandina*, is supposed to have originated through the selfing of a half-mutant called *problandina*, in which the *velutina* gamete had lost its lethal. While the other 3 possess modified *laeta* complexes, therefore, *blandina* contains modified *velutina* complexes.

I have examined cytologically 3 of these half-mutants, and 3 of the homozygous races derived from them. Of the former, *rubinervis* and *erythrina* have a circle of 6 chromosomes, and 4 pairs, and *rubricalyx* has a circle of 8, and 3 pairs. The 3 homozygous races, *deserens*, *latifrons* and *blandina*, all show an entire absence of circles. This is to be expected, of course, if pairing of homologous chromosomes in *Oenothera* means that they are relatively or even entirely homozygous.

It may be possible to explain the origin of half-mutants and their derivatives also on the basis of irregularities in the zigzag arrangement during metaphase. We will take as an example the origin of *rubrinervis* and *deserens* from *Lamarckiana*.

Let us call the $A-F+G$ complex *laeta*, and the $A'-F'+G$ *velutina*. We will now suppose an irregularity in a certain spore mother cell of the type figured in Figure 6. As a result of this irregularity, the members of 3 chromo-

some pairs have become transferred to the wrong complex, providing it occurs at such a point that homologues are not carried to the same pole. The complexes formed when the irregularity takes place at the point indicated in the figure are then $\frac{\text{A B C D}'\text{E}'\text{F}'}{\text{A}'\text{B}'\text{C}'\text{D E F}}+\frac{\text{G}}{\text{G}}$ · Let us suppose that the *velutina* lethal resides in that part of the complex which is not transferred, and that the *laeta* lethal lies in that part which does. Complex ABCD′E′F′G is then free from lethals. Upon uniting with a normal *velutina* gamete, a zygote is formed with the following constitution, $\frac{\text{A B C}}{\text{A}'\text{B}'\text{C}'}+\frac{\text{D}'}{\text{D}'}+\frac{\text{E}'}{\text{E}'}+\frac{\text{F}'}{\text{F}'}+\frac{\text{G}}{\text{G}}$ · D′, E′, and F′ will pair, being identical in the 2 complexes, and hence compatible; so that a circle of 6 and 4 pairs, instead of a circle of 12 and 1 pair, is the result. Again, recessive genes in D′, E′, and F′, which in *Lamarckiana* could never function, may now assert themselves and the plant will have certain new characters of a recessive nature, distinguishing it from *Lamarckiana*. Futhermore, the *velutina* complex of this plant has a lethal, but the mutated complex, which we will call *deserens*, does not. All of these specifications conform exactly to the situation known, through the work of de Vries, to exist in *O. rubrinervis*. When selfed, its 2 gametes will recombine in the following fashion; *deserens*+*deserens*=*deserens*, 25 per cent; *velutina*+*velutina*=dead seeds, 25 per cent; *velutina*+*deserens*=*rubrinervis*, 50 per cent. The complexes of the new form will be as follows $\frac{\text{A}}{\text{A}}+\frac{\text{B}}{\text{B}}+\frac{\text{C}}{\text{C}}+\frac{\text{D}'}{\text{D}'}+\frac{\text{E}'}{\text{E}'}+\frac{\text{F}'}{\text{F}'}+\frac{\text{G}}{\text{G}}$ · All of the homologous chromosomes are identical and compatible, and hence will pair, and no circle will be formed. Recessive genes in chromosomes A, B, and C may now assert themselves, giving to the new form, *deserens*, its distinctive characteristics.

Thus it is seen that there is at least a possibility that the various de Vriesian mutations may owe their appearance to irregularities in the zigzag arrangement of chromosomes in the heterotypic metaphase. Gene mutations, or premutations, as de Vries calls them, may occur from time to time in the chromosomes, but until irregularities in the zigzag arrangement and distribution of the chromosomes release one or more of them from the complexes to which they belong, those germinal changes which are recessive in nature must remain hidden.

Summary

(1) A majority of the original species of *Oenothera* so far studied possess a large number of unpaired chromosomes, which are grouped into circles in diakinesis. These races are all "complex-heterozygotes," that is, they are heterozygous for the single large linkage group. Furthermore, because of their balanced lethal situation, they are permanent heterozygotes, breeding true to type.

(2) Of the races which have been proved to be entirely or nearly homozygous, all so far studied have shown perfect pairing in diakinesis, or at most, a circle of 4. These forms are alethal. Strains intermediate between these 2 classes have shown circles of moderate size.

(3) The circles remain intact throughout the heterotypic metaphase, and the chromosomes comprising them are separated in the anaphase according to the following rule: Adjacent chromosomes pass to opposite poles. A certain percentage of irregularity has been observed in all cases, these irregularities, however, being of certain definite types.

(4) The linking of unpaired chromosomes into circles, and the passing of adjacent chromosomes into different daughter nuclei is a sufficient mechanism to account for the binding of most, if not all, of the genes into a single linkage system, providing the following 2 working hypotheses are correct:

(a) The chromosomes making up the circles have definitely fixed positions with reference to one another, such that, with adjacent chromosomes passing to opposite poles, disjunction occurs between all homologous chromosomes, and the same chromosomes pass together to the poles in all cells.

(b) Most, if not all, of the heterozygous allelomorphs are to be found in the chromosomes comprising the circles, their inability to break from the spireme and to pair being an expression of a high degree of heterozygosis.

(5) Crossing-over may be of 2 types, the ordinary intrachromosomal type, and a sort peculiar to the Oenotheras called interchromosomal crossing-over. The latter is the mutual transfer of one or more entire chromosomes from one complex to the other, and is brought about as a result of occasional irregularities in the distribution of chromosomes in the heterotypic anaphase.

(6) Irregularities such as these may also cause non-disjunction in the case of one or another of the chromosomes, at times resulting in, or accompanying, probably, the formation of the various trisomic mutants. They may also account for the appearance of other types of de Vriesian mutations, such as the so-called half-mutants and the homozygous strains derived from them.

It would seem, therefore, that the peculiar genetical situation in the Oenotheras has as its basis an equally peculiar type of chromosome behavior in meiosis. A continued study of *Oenothera* cytology should yield inportant returns.

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INFLUENCES DETERMINING THE APPEARANCE OF SEXUAL CHARACTERS[1]

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The word *character* is here employed in its usual meaning, that of a quality for convenience considered separately. We treat as a character the production of a stamen, or its division into filament and anther, or the formation of microspores, or the germination of a pollen grain. Each such character is an artificial abstraction for the purpose of the immediate discussion; it is very different from the "unit characters" of a now obsolescent theory. Any "character" of a cellular aggregate is of course a summation of characters of the constituent cells.

In a comparable way, all conditions that contribute to or affect the appearance of particular characters will be spoken of as factors. In this sense the word *factor* is both more inclusive and less specific than in its familiar genetic usage.

The term *sexual* is commonly made to cover characters of very diverse sorts. It is important that characters belonging to different categories be clearly distinguished. A classification of characters, *as characters*, must rest upon their physiological and evolutionary significance. That characters thus assigned to distinct categories may be functionally interdependent or genetically interrelated is another story.

One category includes substantially those structures and functions that to common perception distinguish male and female cells, organs, and organisms. Characters of this class appear in evolutionary history when the gametes of a pair, previously similar, begin to differ visibly in size, in behavior, or in both. This differentiation accompanies a degree of separation between two functions that the gametes must perform; namely, insuring mutual contact, and providing a food-supply for at least the temporary use of the zygote. Since food-storage is favored by greater size of gamete, and since motility, insuring contact, is favored by lesser size, a separation of the 2 functions is of advantage to the species.

If the zygote becomes a resting cell, or if the developmental history of the generation arising from the zygote comes to include a juvenile or embryonic stage, the importance of a food-supply is increased. Further steps in sexual differentiation include the production of special organs within which the respective gametes are differentiated; and in some lines of descent, a separation of the functions of gamete-production between distinct male and female organisms. Provision for food-supply, enabling a prolongation of embryonic development, necessitates also provision for the shelter or protection of the embryo. Approxi-

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mation or contact of gametes may be effected by copulatory organs or pollen tubes; and the female gamete may be largely relieved of the duty of food-supply through the development of various devices by the female organism. In seed plants, means to insure gametic contact and embryonic nourishment and protection are developed by the generation grandparental to the embryo; sexual characters thus appearing in an originally non-sexual generation.

The greater part of the characters commonly recognized as sexual have, therefore, develcped in reference to the needs of gametic contact on the one hand, and on the other, of food-supply, and secondarily protection, for the zygote and the offspring. Meeting these contrasting and conflicting needs, the characters in question inevitably display a "bipolarity"—in the sense that they represent two opposed developmental tendencies. Bipolarity does not here imply the existence of maleness and femaleness as distinct or mutally exclusive qualities.[2]

To a second category belong certain characters that favor, hinder, or regulate the occurrence of syngamy. In seed plants, the possibilities of self-, close-, or cross-pollination and of subsequent syngamy depend upon characters of both gametophyte and sporophyte. Dichogamy and heterostyly are illustrations. The distribution of certain other characters of this category may be such that, as in *Nicotiana*, a species includes several or many classes, characterized by group intra-sterility[3] and inter-fertility. The characters that distinguish such classes are obviously distinct from characters of the previous category. Not only have they, and other characters of the second category, no necessary relation in occurrence to maleness or femaleness, they frequently differ also from those of the first category in manifesting a *multipolarity*.

Organisms of other groups—animals (*Ciona*) and algae (*Ectocarpus*)—likewise display characters of the second category. In certain Basidiomycetes showing little or no sexual differentiation, the mycelia (or conidia) constitute distinct classes, as shown by their ability or inability to conjugate one with another. Since of such classes there may be within a single species 2, 4, 20, or more, the distinguishing characters show a multipolarity. Those who treat the differences between such classes of mycelia as sexual are compelled

[2] The first category of characters as here defined is of course capable of subdivision. "Primary" and "secondary" sexual characters represent one possible segregation, which might be useful were the distinction between primary and secondary agreed upon. Prell prefers to classify sexual characters (substantially those belonging to this first category) according as they are manifested by haplonts or by diplonts. Such a criterion logically applied would lead to such absurdities as a separation between the sexual characters of a haploid and those of a diploid moss gametophyte. It would also necessitate new classes and designations for the sexual characters of triploid, tetraploid, and all other possible polyploid and heteroploid types. It may be noted, too, that Prell in his classification uses both genotypic and phenotypic criteria. The viewpoint of the present paper is that, characters being phenotypic phenomena, their grouping for any practical purpose must rest upon phenotypic considerations alone. That a classification of characters based upon genetic relationships is possible, given adequate knowledge, is not to be denied. The linkage groups of *Drosophila* may illustrate the nature of such a classification.

[3] "Sterility" in the sense of a failure to develop embryo, seed, or fruit may result from causes operating after syngamy has occurred. Such phenomena are apart from the present discussion.

to recognize the possibility of an indefinite number of "sexes." From a similar point of view, Kniep speaks of 2 mycelia or conidia that can conjugate as showing a "physiological sex-differentiation." Now, in the usual meaning of words, the difference in behavior between male and female gametes of *Spirogyra* involves a "physiological sex-differentiation"; but the characters that regulate conjugation between similar and similarly functioning cells of *Aleurodiscus* determine a difference, if it is a difference, of another sort from that between the gametes of *Spirogyra*. If one is a case of physiological sex-differentiation, the other is not.

The distinction in many Mucorineae between plus and minus mycelia, although multipolarity is not here demonstrated, appears likewise to rest upon characters of the second category. Some Mucorineae also display structural differentiations between gametes and gamete-bearing branches, which seem to correspond to character-differences of the first category. *Absidia spinosa*, a homothallic species with differentiated gametes and progametes, forms small (male) gametes in contact with a plus mycelium of a heterothallic Mucor, and large (female) gametes in contact with a minus mycelium. Blakeslee concludes from these facts that minus mycelia are male and plus mycelia female. On the other hand, in *Rhizopus nigricans*, which is heterothallic, the gametes (and progametes) of a pair commonly differ in size. Blakeslee finds that the larger or the smaller gamete may be borne indifferently upon either plus or minus mycelium, and, thinking of the plus-minus differentiation as sexual, he holds logically that "the inequality between conjugating gametes has no necessary relation to sex." But it is awkward, to say the least, to think of size of gametes as a sexual character in one species and as not a sexual character in a related species; and one may say with equal force that, size of gametes being considered a sexual character, plusness and minusness have no necessary relation to sex. Difficulties of this nature vanish with the recognition that characters of distinct categories are in question; although, as in *Absidia*, those of 2 categories may be related in inheritance.

Another class of still questionable characters must be mentioned. Delicate chemical tests indicate the frequent but not constant occurrence of differences, of similar order in various plants and animals, between male and female individuals as well as between the sexually differentiated organs and cells of a bisexual individual. The discovery of similar chemical differences between visibly similar gametes, if made, would strengthen the popular notion that gametes united originally because of some protoplasmic difference. Although with no basis as yet in observed fact, such gametic differences primitively necessary to syngamy have been so often postulated that the erection of a third category to include them may be justifiable. Such distinctions being thought of as sexual, the term "sexual reproduction" has been applied to the union of like gametes, with resultant sad confusion. Similar confusion underlies much of the discussion of "sexual states." Characters of this third category, if they exist, resemble those of the first category in the quality of bipolarity. They differ in that, still assuming their reality, they existed before, and were prerequisite to, the occurrence

of syngamy; whereas characters of the first category represent responses to needs that arose in consequence of syngamy. The chemical characters now found to distinguish some male and female structures or organisms may evidence a persistence of the supposed primitive differentiating characters; they may mean something quite different. In the former case, it would not be surprising that their occurrence coincides frequently with that of characters of the first category. That they may also coincide in occurrence with characters of the second category (plusness and minusness) is indicated by tests of Mucorineae.

Current discussions are marked by a lack of clearness caused by failure to distinguish these three categories. The need of distinctions more or less like some of those here attempted has been variously urged; notably, very recently, by Prell, Brunswik, and Morgan. If we are to escape confusion, the meaning of the word "sexual" must be sharply limited. Here, it will be applied to characters of the first category; a usage most nearly in harmony with the general and long-established one. Sexual characters are defined, then, as those that arose in adaptation to the needs, imposed by the occurrence of syngamy, for securing the contact of gametes and for insuring the sustenance and protection of the zygote or of the juvenile offspring. They do not include any characters which may have been phylogenetically antecedent to syngamy; nor do they include any, whenever they arose, which favor or hinder the union of gametes otherwise capable of functioning.

Sexual characters so defined include not only the development of sexual structures and functions; they include also characteristics of time, place, and frequency of appearance of such structures and functions. For example, monoecism and hermaphroditism, and the various other possible arrangements of stamens and carpels in seed plants, or of antheridia and archegonia in mosses, are sexual characters, distinguishing genera, species, races, and strains, as well as individuals of the same strain under differing conditions. Further, structure and function may be separated; functionless or rudimentary organs characteristic of 1 sex often appear in individuals that are functionally of the opposite sex. The presence of a rudimentary carpel is a female character although the plant bearing it is functionally male. Conversely, male types of behavior may characterize structurally female animals. Hence a discussion of the causation of sexual characters properly covers much more than the determination of a simple alternative between maleness and femaleness.

Certain facts with a general bearing may be taken as established. These need only be stated; they have been recently and adequately discussed by other writers.

First, sexual characters are not distinguishable as to mode of determination from other characters. The appearance of female structures in one part and of male structures in another part of the same plant is a phenomenon of the same order as the appearance of leaves in one region and of roots in another. The explanation of the 2 phenomena is to be reached by a similar method of attack. Likewise, the occurrence of male and female organs in separate individuals of a

race is a phenomenon of the same order as the occurrence of white and blue flowers on separate but otherwise similar and interfertile plants.

Second, influences affecting the appearance of sexual as of other characters are of 2 sorts. Each organism possesses a range of developmental potentialities which inheres in each of its cells; what of these potentialities are realized, and to what extent, depends upon the conditions surrounding the organism during its development. The visible character of the individual is, then, determined by the joint action and interaction of genetic and environmental factors. This fact is simple and obvious. Its general comprehension as related to sexual characters would obviate much controversy that calls to mind the ancient tale of a cooperative research project bearing on the external morphology of the elephant.

Third, any factor, genetic or environmental, is related to the appearance of a particular character through a long series of intermediate processes. Let us speak of such series of processes as *genetic chains* and *environmental chains*. Each process in a chain of either sort is an effect of preceding, and a cause of succeeding, processes.

Fourth, the processes of distinct chains may interact, with consequences of acceleration, retardation, inhibition, or modification. Processes in 1 chain thus become causal to succeeding processes in another chain. The total result can be visualized as a network of bewildering complexity, by whose means each genetic or environmental factor is related as a cause to many or all of the characters of the individual; and each character is related as an effect to many or all of the factors, genetic and environmental.

Another conception, while not a demonstrated fact, is firmly established as a working hypothesis. This is, that characters which show a Mendelian, or approximately Mendelian, distribution are differentially affected by factors whose genetic bases are carried by the chromosomes. It is recognized, of course, that the cytoplasm is also the seat of genetic factors, at least to the extent that it is necessary to the functioning of substances borne in or on the chromosomes; and that it seems to contain some genetic factors demonstrably differential for certain characters.

In a study of the causation of sexual characters, it is important to know what range of characters of this class is made possible by the genetic constitution of a given individual. Ideally, the solution of this problem would require the subjection of the organism to every combination of environmental conditions that it can endure. In practice the requirement could be approximately met by subjecting the individuals of a clone, all presumably alike genotypically, to a wide variety of conditions. Extensive experimentation of this nature remains for the future. What we know at present is something of the range of possibilities among the members of a species or race; and chiefly with reference to dioecism or bisexuality—that is, to the possibility of expression of maleness or femaleness or both, considered, rather vaguely, as wholes.

It appears from information thus gained that in the majority of bryophytes each haploid gametophyte is potentially bisexual. A considerable number of

species are classed as "dioecious"; but in relatively few has strict dioecism been demonstrated. The latter include several species of mosses investigated by the Marchals and later workers; and, among hepatics, *Marchantia polymorpha*[4], *Conocephalum conicum*, and three species of *Sphaerocarpos*. So far as present evidence goes, each haploid individual of these species can produce male or female, but never both male and female, structures. That this is not and perhaps never can be finally proved for any species goes without saying. So far as known, the sporophytic generation of a bryophyte never displays sexual characters; but that it possesses sexual potentialities is shown by the production from it through apospory of a (usually) bisexual gametophyte.

In homosporous pteridophytes the gametophyte seems always to be potentially bisexual. The sporophyte displays no sexual characters. In heterosporous pteridophytes and in seed plants, the gametophytes are always, so far as known, respectively strictly male or strictly female. What further capacities they may possess remains to be determined.

In heterosporous pteridophytes and in the majority of seed plants, the sporophyte is bisexual. Some gymnosperms seem to be dioecious, but experimental evidence is lacking on this point. While many angiosperms are classed as dioecious, it seems to be true that, in every species that has been intensively studied in this respect, each sporophyte possesses both sets of sexual potentialities.

In most metazoan groups, functional unisexuality is so nearly universal as to suggest that in general a male is by genetic necessity male and a female just as necessarily female. But certain facts suggest a doubt upon this point. First is the fundamental difference in method of development between metazoa and angiosperms. If an angiosperm were limited to the production of 1 set of functional sex organs and the rudiments of these were formed at an early embryonic stage; or, if an animal could form, from a meristematic region, new sets of sex glands during the period of its active life, the differences in sexual expression between so-called "higher plants" and "higher animals" might well be less conspicuous. Further doubt is cast by the frequent occurrence in animals of one sex of rudiments of the organs of the opposite sex; by the occurrence of cases of intersexuality; and by a few reported instances of complete sex reversal.

It is not certain, therefore, that any sexually differentiated animal or plant is limited genetically to the production of male or female characters only; although in many animals and in some bryophytes the present evidence points to the existence of such a limitation.

Even less is known of the ranges of possibility as to the frequency of appearance or the localization of sexual structures and functions. The sexual structures in bryophytes, and in the sporophytic generation of pteridophytes and seed plants, may under some conditions be more numerous and under others less numerous or entirely lacking. In *Funaria hygrometrica*, the distribution of antheridia and archegonia varies with the environment. Intersexual structures

[4] So far as disclosed by a careful search of the literature bearing on this much-studied species. Certain observations of Cutting on plants of 1 or 2 other species of *Marchantia* are often erroneously cited in this connection.

have been observed on potentially bisexual mosses and angiosperms. In angiosperms, apart from changes from a chiefly staminate to a chiefly pistillate character and *vice versa*, variations occur from hermaphroditism to andro- or gynomonoecism and the like. Such instances suffice to show at least a widespread plasticity as concerns the frequency and the distribution in time and space of one or both sets of sexual structures.

Next comes the problem of the nature of the genetic factors which affect the appearance of sexual characters.[5] It is not to be expected that the solution of this problem will be simple or that 1 solution will fit all cases. In this connection, the discovery of a chromosomal difference between the sexes in certain insects made possible the first step in the analysis of inheritance in terms of individual chromosomes. We now know that in many animals, in a considerable number of angiosperms, and in 1 genus of hepatics, 2 individuals differing usually or constantly (under similar conditions) in their sexual characters differ also in their chromosome complements. In these organisms, therefore, a causal relation exists between sexual characters and the presence of particular chromosomes. It follows that the substances, or some part of the substances, borne in or on these chromosomes are among the genetic factors that influence the appearance of sexual characters.

This does not necessarily mean that the differences in chromosome size are themselves thus causally related. In many cases the larger amount of chromosomal material is correlated with the appearance of female characters; but in certain instances the reverse is true. In many animals and plants, equally high degrees of sexual differentiation accompany no recognizable differences between the chromosomes of the 2 sexes. It has been suggested that in *Sphaerocarpos* the difference in size between the X- and the Y-chromosome may affect the differences between the sexes in rate of growth and cell division, and consequently in size of plant, rather than the differences between sex organs or gametes. The greater size of the female plant seems to be related to its function in the nourishment of the offspring; it is, therefore, perhaps to be classed as a sexual character. This notion of the possible significance of chromosome size is supported by the fact that in other dioecious hepatics (*Conocephalum*, *Riccardia*, and *Porella*) in which the sexes differ little or not at all in rate of growth and cell division no chromosomal difference has been detected. Possibly the most that can now safely be said on this point is that in many organisms the chromosomes recognizable by their comparative sizes bear factors differential for sexual characters; and that in some instances chromosomal size differences may themselves be related to certain possibly sexual distinctions, namely, those in the rate of processes underlying growth and cell division.

Apart from the factors that consist in, or are intimately correlated with, visible differences in chromosomes, there are other factors affecting sexual characters which are separable in inheritance from those just mentioned, and variations in which are not accompanied by perceptible (or at any rate perceived)

[5] The factors here discussed may, of course, also influence many characters that, as characters, are unrelated to sex.

chromosomal differences. Clear-cut evidence for the existence of such factors is polyploid and heterploid Drosophilas. In maize at least 8 factor-pairs are recognized which correspond to differences in localization of male or female structures. Hermaphroditic plants of *Lychnis* whose genetic behavior indicates that they are modified males, as well as thelygonous males, have been found to possess the chromosome complement characteristic of plants which, similarly situated, are ordinary males. The genetic differences in these instances depend upon unperceived differences in the chromosomes. Also, but conversely, individuals of the hermaphroditic generation of *Angiostomum* have the chromosome complement characteristic of females of the sexual generation. Three races of *Sphaerocarpos Donnellii* are known which differ from a typical race in bearing, under like conditions, a lesser proportion of sexual structures, as well as in certain certain other respects. The females of 2 of them are functionally less fertile than the type; 1, indeed, is entirely sterile. No differences have been found in the chromosomes of these races when compared, sex for sex, with a typical race.

Some of these factors that are not necessarily correlated with visible chromosomal characteristics may be borne on the allosomes; some are certainly borne on autosomes. This latter is the condition in *Drosophila melanogaster*, at least two of whose three pairs of autosomes are concerned with sexual expression. In maize, the independent or linked behavior of the 8 factor pairs referred to relates them to the chromosomes of several distinct pairs. In *Sphaerocarpos* it is highly probable that the characters of the atypical races mentioned find their genetic bases in chromosomes other than the X or the Y.

Experimental evidence of linkage relations and cytological evidence agree in indicating that, as Roux held long ago, chromosomes are longitudinally differentiated. It is true that there are as yet few indications as to the intra-chromosomal localization of sex-influencing factors. But if we adopt the idea of a linear differentiation of the hereditarily effective substances of the chromosomes, then it is probable that the substances influencing the development of sexual characters must in general be similarly localized. Such localization seems to be established for certain sex-influencing factors in maize and for one factor in *Drosophila simulans*. The possible influence of merely quantitative chromosomal differences upon such characters as differential rate of growth may be an exception to the general rule. Our conception of the genetic sex-influencing factors, then, is in the main that of qualitatively different substances distributed along the chromosomes, both autosomes and allosomes when the latter are recognizable.

This conception has the disadvantage of the vagueness that inheres in a statement based on ascertained facts. The conception becomes easier to deal with if for factors in the broader sense we substitute those discrete material entities or units that at present are commonly referred to as "genes." To the geneticist the gene-concept is extremely useful. It meets, for the moment, all the requirements of a valid hypothesis. But, as has happened to other useful hypotheses, that of the gene suffers from being treated as a dogma. The attitude of a considerable class of biologists toward the concept of the gene is that of a fundamentalist toward the doctrine of the virgin birth. To a cytologist, not

needing hypothetical units in his business, the important question is one of fact: is there valid evidence of the real existence of structures of the nature of genes?

One who has dallied with the possibility that chromomeres might represent Mendelian units or nests of such units may now confess that such a notion finds no cytological support. Chromosomes of different species, studied at different stages and by different methods, display a differentiated core, spiral or approximately parallel to the long axis of each chromosome; two internal spiral strands, differentiated zigzag bands, crosswise bands, separate granules, or alternating discs. Which of these appearances most nearly represents a fundamental structure, or whether the fundamental organization varies from stage to stage or from species to species, is still uncertain; although, as already noted, these observations, together with those of localized constrictions, demonstrate the existence of some intrachromosomal differentiation. That the varying substances of the chromosomes are organized into discontinuous units of biological significance is a notion without observational basis.

The sole argument in favor of such a conception is that the gene-hypothesis works. On the other hand it is true that almost any imaginable state of genetic facts can be expressed in terms of genes provided we are indefinitely free to subdivide old genes and invent new ones. It is true also that the analysis of determined facts inevitably proceeds, in the inductive sciences as in mathematics, by the division of observable realities into unobservable and hypothetical units. This tendency, illustrated in the physical as well as in the biological sciences, may throw important light unpon the genotypic background and the phenotypic organization of the human mind; but that very fact suggests grave doubt as to the reality of the postulated units. One may recall the great value of the atomic hypothesis during the last century, as well as the fact that the nineteenth-century atom, like the genetic units of the early twentieth century, has quite disappeared; or that the properties of a cube may be demonstrated in terms of points, lines, and planes, although points, lines, and planes as mathematically defined are non-existent. It is significant, too, that the gene-concept has not been fitted into what is known of the physico-chemical constitution of living matter. Perhaps the most nearly successful attempt of this nature is the suggestion that a gene may be an organic molecule; and this suggestion is likely to appeal the more strongly to a biologist because he may not realize the purely hypothetical nature of a molecule.

Plainly, the usefulness of the gene-hypothesis is not evidence of a gene-reality. Cytologists must be content with the conception, safer though less easy to state in concrete terms, of significant physico-chemical differences between different chromosomes as well as between different regions of the same chromosome. To this extent the substances of the chromosomes are localized; and some of these more or less definitely localized substances are among the genetic factors upon which sexual and other potentialities depend.

The whole chain of processes beginning with a genetic factor and ending with the appearance of a particular character may conceivably operate within a single cell. This is especially suggested in the study of a plant, each of whose

cells runs the whole course from an embryonic condition to whatever degree of differentiation that cell achieves. The development of any cell, however, seems to be influenced by the course of development followed by its immediate ancestors and by its sister and cousin cells. Thus the initiation of a stamen, if its development is not disturbed or checked, regularly leads to the development of filament and anther, of microspore mother cells and microspores. Apparently a genetic chain operating within 1 cell may affect a distinct chain operating in another cell; and by such means, through a series of intercellular stimuli, a factor, initiating a genetic chain in 1 cell, may influence the characters of cells long subsequent in time of appearance. In the more complex metazoa, in which differentiation extends over a long series of cell generations, the interaction between originally distinct genetic chains, and the integration of a complex chain or network of processes extending through many cell generations, become more evident.

Similarly, an environmental chain may interact with a genetic chain or network to influence the appearance of sexual or other characters. Thus environmental factors may favor or inhibit the appearance of sexual structures; they may influence the degree of development, the abundance or scarcity, the functional efficiency, and the localization of such structures; or they may divert the trend of development at almost any point from sexual to non-sexual, from male to female, or from female to male. But environmental factors can influence the expression only of potentialities genetically present. If a once pistillate plant is induced to produce staminate flowers, the result shows that genetic factors are present which make possible the production of stamens.

A further problem, then, is to determine what environmental factors may affect sexual expression. Experimental study of angiosperms has turned largely upon the environmental conditions that either affect the numbers of sexual structures produced or modify the sexual expression of individuals that are ordinarily either chiefly staminate or chiefly pistillate. Among external conditions that seem to have the latter effect are mechanical injury, mutilation, or pruning; spacing or crowding; the length of the daily period of illumination; altitude and temperature (possibly); depth, water-content, and chemical constitution of the substrate, especially its content in organic nutrients and nitrogen; and the amount of food-supply in storage organs. The last-named is a factor internal to the plant, doubtless dependent upon some of the factors before mentioned and therefore representing a step in 1 of the chains of causation which lead to the effects observed; but a step still far removed from the cells immediately concerned in the production of sexual characters. Other intimate factors are infection by a parasitic fungus, and the carbohydrate-nitrogen ratio within the plant. "Within the plant" may here imply within the organs and cells that become sexually differentiated, or it may not; the direct operation of such internal factors may be upon parts of the plant distant from those in which the ultimate effects are observed.

Factors of the same order are some of those just mentioned are known also to affect the proportion of sexual structures borne by angiosperms (thus deter-

mining "fruitfulness"); as well as the numbers and proportions of male or female structures borne by mosses and by fern prothallia.

Environmental factors of another sort are illustrated by Hartmann's study of conjugation in *Ectocarpus*. Flagellate cells produced by one plant, at least, in some instances, are genetically capable of functioning as either male or female gametes. The alternative is determined by the presence of flagellate cells from another plant. Those non-sexual properties of the second plant and of its motile cells which thus, as environmental factors, affect the sexual character of cells from the first plant probably belong among the characters of our second category.

Among strictly external factors found to influence the development of sexual characters in metazoa are temperature, relative abundance and nature of food (including that stored in the egg), water-content of the egg, free-living, attached or parasitic mode of life, the presence or absence of a larger individual of the same species, crowding, amputation of part of the body, and infestation by a parasite. These are comparable with the external factors that affect sexual characters in angiosperms, and in metazoa as in angiosperms few hints appear as to the nature of the series of processes through which external conditions produce their ultimate effects. There is a tendency to explain the action in both plants and animals of factors like those mentioned by their influence upon nutrition ("rich" nutrition favoring femaleness) or upon metabolism (the 2 sexes being characterized by different "metabolic levels"). These explanations, if valid, may constitute a short step toward the analysis of environmental chains. It is quite possible, however, that some cases belonging in the classes mentioned involve changes in the chromosome complement of the organism, the effects of external conditions thus being produced directly upon the complex of genetic factors.

Certain other influences that affect the sexual characters of animals are explainable by their influence upon or through hormones. Possibly the effects of 1 or 2 factors already mentioned are thus to be explained; as are those of castration, ovariotomy, testicular or ovarian tumors, transplantation of sex glands, and the presence of a male twin, the last-named condition resulting in the development of a freemartin. Such instances, accepting the explanation, involve a modification by external means of a genetic chain one step in which is the production of a hormone; and, in the cases of transplants and of freemartins, a substitution of the latter part of a different genetic chain, with the result that some of the characters appear to which the substituted chain naturally leads.

As to both genetic and environmental factors concerned in the appearance of sexual characters, a good deal is thus known of the nature of the factors themselves, and of the end results of their action. Here and there a suggestion appears as to the nature of some of the long chains of processes that connect a factor of either type with a particular character; as to the way in which one chain of causation may interlock with another chain; and of how and when the network of genetic chains may be acted upon by and interlock with the network of environmental chains.

THE CYTOLOGY OF TRITICUM IN RELATION TO GENETICS[1]

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Among the various botanical sciences represented at the International Congress of Plant Sciences, genetics and cytology are undoubtedly more closely associated and interdependent than any of the other groups. Cytology has been a valuable aid in interpreting genetic problems. The segregation of genetic factors, the determination of sex, and many types of mutations have been found to be closely associated with chromosome behavior.

The recent cytological work with wheat offers one of the best examples of the value of cytology as an aid in the solution of genetic problems. The international scope of this work is also of considerable significance, since cytological investigations with *Triticum* have been reported from Japan, Canada, England, Poland, Holland, Germany, Denmark, Austria, Russia, and the United States.

Sakamura was the first cytologist to report correctly the chromosome numbers in the cultivated species of wheat. The Einkorn group, containing the single species *T. monococcum*, has 7 haploid chromosomes. The Emmer group, consisting of *T. durum*, *T. dicoccum*, *T. turgidum*, and *T. polonicum* has 14 haploid chromosomes. The Vulgare group, consisting of *T. Spelta*, *T. compactum*, and *T. vulgare*, has 21 pairs of chromosomes. During the past 5 or 6 years these counts have been verified by a number of investigators in various parts of the world. The classification of wheat species according to chromosome number is in accord with Tschermak's and Percival's taxonomic division, with Zade's serological relationships, with Vavilov's classification in regard to disease resistance, and with sterility relationships in species crosses.

The chromosome number of closely allied genera is also of interest in connection with generic crosses and the possible origin of wheat species through hybridization. According to Sakamura and other investigators rye has 7 pairs of chromosomes. Both *Aegilops ovata* and *A. cylindrica* have 14 haploid chromosomes. The species of oats have 7, 14, and 21 pairs of chromosomes. The cultivated species of barley have only 7 chromosomes although some species of no economic value have 14 pairs of chromosomes. In all of these cereals 7 appears to be the basic chromosome number.

The species of wheat with 7, 14, and 21 chromosomes can be crossed with each other although the F_1 hybrids are more or less sterile. A cytological study

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of such hybrids has shown the reason for such sterility and has indicated the chromosome relationships of the various species. In the cross of Einkorn with a variety of *T. turgidum* the F_1 plant has 21 somatic chromosomes, 7 from one parent and 14 from the other. At the time of the first reduction division of the pollen mother cells there are about 7 pairs and 7 single chromosomes. The 7 Einkorn chromosomes presumably pair with 7 of the Emmer chromosomes, leaving 7 single chromosomes contributed by the Emmer parent. The paired chromosomes divide normally. The singles do not usually divide but pass to, or remain at, the poles of the spindle without dividing. The distribution of the single chromosomes to the daughter cells seems to be at random. In the second division all of the chromosomes divide, and except in cases where chromosomes are lost the pollen grains receive from 7 to 14 chromosomes. The tetrads appear to be normal in most cases, but only about 2 per cent of the F_1 pollen develops completely and even these pollen grains are probably functional only in rare cases. Sterility even in the female gametes is almost complete.

In crosses between members of the Emmer group and members of the Vulgare group of wheat species the F_1 plants have 35 chromosomes, 14 from the Emmer parent and 21 from the Vulgare parent. During the first reduction division there are 14 paired and 7 single chromosomes. The single chromosomes do not become oriented on the spindle until the bivalents have divided. The lagging univalents are very conspicuous in these division figures. They ultimately divide lengthwise, however, and pass to the poles with the members of the bivalent pairs.

In the second division the members of the original bivalents again divide normally but the 7 univalents are distributed to one pole or the other without dividing. The univalents are distributed to the daughter cells at random so that the pollen grains receive 14 chromosomes from both parents according to chance and from 0 to 7 chromosomes from the Vulgare parent. Thus the F_1 gametes have from 14 to 21 chromosomes, the numbers of each class depending on chance distribution. The one-nucleate microspores appear to be normal, but about 20 per cent of the F_1 pollen is obviously aborted, and a much larger percentage is undoubtedly non-functional.

In the second generation of crosses between Emmer and Vulgare wheats all degrees of sterility are found. Some seeds do not germinate; others germinate but do not develop; many plants form heads but are more or less sterile, while only 1 or 2 per cent of the F_2 segregates are as fertile as the parents. If all F_1 gametes are functional, the parental chromosome numbers should be recovered only once in about 16,000 times. A cytological study of F_2 and F_3 segregates shows that a very large proportion of the plants have either 28 or 42 somatic chromosomes. Moreover the ultimately fertile homozygous segregates have only 14 or 21 pairs of chromosomes and closely resemble the parental types. These conclusions have been substantiated by Kihara, by Watkins, and by Thompson. Kihara and Watkins have in addition shown that all segregates with less than 35 chromosomes have only 14 bivalents at reduction, while in segregates with more than 35 chromosomes the sum of the number of bivalents and univalents is always equal to 21.

Crosses of different genera of cereals are of value in determining genetic relationships. The crosses of *Aegilops* with wheat are especially interesting because *Aegilops* is supposed to be involved in the origin of the Vulgare wheats. Both *Aegilops ovata* and *A. cylindrica* have 14 pairs of chromosomes. When *A. cylindrica* is crossed with one of the Vulgare wheats the F_1 plant contains 14 chromosomes from *Aegilops* and 21 chromosomes from the wheat parent. At the reduction divisions there are about 7 pairs of chromosomes and 21 single chromosomes. Apparently 7 of the *Aegilops* chromosomes pair with 7 wheat chromosomes, leaving 7 singles from the *Aegilops* parent and 14 singles from the wheat parent. The bivalents divide normally, but the univalents are apparently distributed at random to the pollen grains so that the gametes contain from 7 to 28 chromosomes. Sterility is almost complete although a few grains are occasionally found on the F_1 plants.

Since 7 *Aegilops* chromosomes pair with 7 of the Vulgare chromosomes a similar pairing might be expected when *Aegilops* is crossed with a 14-chromosome wheat. Gaines has recently found, however, that when *Aegilops ovata* is crossed with an Emmer wheat there is no pairing of the chromosomes in the reduction divisions of the F_1 hybrid. Twenty-eight single chromosomes are present and the division is very irregular. The F_1 plants are completely sterile. During the past summer I have found similar results in a cross of *A. ovata* with a durum wheat. Unfortunately a cytological analysis of hybrids between *A. cylindrica* and Emmer wheats has not been made.

Chromosome number and behavior in wheat species and hybrids are of interest from the standpoint of both sterility and genetic relationships. There is also a rather striking association between chromosome number and economic value in wheat species. The Einkorn group with 7 pairs of chromosomes contains only a few varieties and these are of no economic value. The Emmer group with 14 pairs of chromosomes contains about 150 varieties which are of considerable agricultural value, particularly in semi-arid regions. The Vulgare group with 21 pairs of chromosomes is very adaptable. Members of this group are grown in all parts of the world where wheat can be grown. Percival describes more than 500 varieties in this group.

The three groups of wheat species also differ greatly in their susceptibility to disease. Most investigators, in Europe at least, have found Einkorn to be highly resistant to rust. The species of the Emmer group are relatively resistant, while the species of the Vulgare group are in general susceptible. A similar relation is found in the susceptibility to bunt and mildew.

The Vulgare wheats are also the only ones possessing the proper quality of gluten for the manufacture of light bread. Thus we find differences in chromosome number to be closely associated with differences in morphological characters, in adaptability to environmental conditions, in disease resistance, and in quality of grain. The reasons for this association are not entirely clear, but it may be attributed to chromosome reduplication or increase in chromosome number through crossing, thereby offering greater opportunity for mutations to occur and permitting the effect of a greater number of genetic factors.

The behavior of the chromosomes in species hybrids indicates rather clearly the primary cause of the sterility relationships. In the crosses of Emmer× Vulgare wheats the amount of sterility in the F_1 plants varies somewhat according to the varieties used. In most cases, however, about 10 to 20 per cent of the F_1 pollen is obviously imperfect. Watkins finds in such crosses that about 90 per cent of such pollen fails to germinate on F_1 stigmas. In back crosses of the F_1 with parental pollen he finds only about 10 per cent of the egg cells to be sterile.

In F_2 all degrees of somatic and gametic perfection are found. Since the fertile segregates have only 14 or 21 pairs of chromosomes it is evident that gametic and somatic perfection increases as the chromosome number approaches the parental numbers and that gametes and segregates with an intermediate number are rapidly eliminated. Since only about 10 per cent of the F_1 pollen grains germinate on F_1 stigmas it is probable that the male gametes with either 14 or 21 chromosomes function most frequently. This supposition is supported by the fact that in segregates with less than 35 chromosomes the number of bivalents is never more than 14 and in segregates with more than 35 chromosomes the total number of the bivalents and univalents is always 21.

Since about 90 per cent of the F_1 ovules are functional with parental pollen most of the chromosome combinations from 14 to 21 are fertile. However, only about 25 per cent of the F_1 ovules set seed when pollinated with F_1 pollen and only about half of the seeds produced develop into F_2 plants which mature. Thus only about 10 per cent of the egg cells produce F_2 plants. Undoubtedly the egg cells with chromosome numbers approximating 14 or 21 function most frequently. With such selective fertilization and elimination of gametes it is obvious that a large percentage of the F_2 segregates will have either 14 or 21 pairs of chromosomes. Since gametes and segregates with chromosome numbers intermediate between 14 and 21 are more or less sterile it is evident that the random distribution of the single chromosomes is responsible for most of the sterility found in these hybrids. Only those gametes and segregates with a chromosome number which is a multiple of 7 function perfectly.

Cytological studies have not only explained the reason for sterility in species hybrids of *Triticum*, but have also made clear certain peculiar genetic results in such hybrids. Plant breeders for many years have attempted to combine the drought and disease resistance of the Emmer wheats with the good bread-making qualities of the Vulgare wheats. Although many thousands of segregates have been grown the desirable characters of the two groups have been combined in a segregate of economic importance only rarely, if at all. The fertile segregates in these crosses are similar to the parental types and combinations of the more typical parental characters are not obtained. This association of parental characters in F_2 and subsequent generations has been attributed to chromosome linkage by several investigators, but the cytological analysis of these hybrids has shown the true reason for their behavior.

Since all 14-chromosome segregates resemble the Emmer parent it follows that the primary 14 chromosomes of the Vulgare wheats are similar in genetic

constitution to the 14 Emmer chromosomes. And since all 21-chromosome segregates resemble the Vulgare parent it appears that the typical differentiating Vulgare characters are dependent on the extra 7 chromosomes which these segregates possess.

In some of the more fertile crosses the 14-chromosome segregates occasionally contain a few typical Vulgare characters and some of the 21-chromosome segregates contain one or more of the typical Emmer characters. The cytological basis for such behavior is not entirely clear, but it is probable that in some crosses certain of the extra 7 chromosomes may pair with some of the 14 chromosomes of the Emmer group and the corresponding Emmer chromosomes may act as univalents in the reduction divisions. In general, however, the parental characters are closely associated in the fertile segregates, and combinations of the typical and valuable characters of these 2 groups of wheat species can rarely be obtained.

If the extra 7 chromosomes of the Vulgare wheats determine the typical characters of the Vulgare group such characters should not be inherited in an orderly manner in species crosses because of the irregular distribution of the univalents and the sterility caused by such behavior. On the other hand, characters common to the 2 groups are presumably determined by the primary 14 chromosomes and should be inherited in the usual Mendelian manner. Genetic investigations have substantiated this theory. In a cross of an Emmer wheat with a Vulgare variety the length of awns, awn color, and grain color segregated in the same ratios that are found in varietal crosses. On the other hand, the characters which distinguish the Emmer and Vulgare wheats do not show Mendelian segregation in species crosses. For instance, in crosses of Alaska wheat with other Emmer wheats the branched character of the spike segregates in a ratio of 3 to 1 while in crosses of Alaska with a Vulgare wheat no typical Mendelian ratios are obtained. The available genetic data support the theory that the extra 7 chromosomes of the Vulgare wheats determine the characters which distinguish them from the Emmer wheats.

A study of chromosome number in wheat species and allied genera, and the behavior of the chromosomes in species and genus hybrids, should throw some light on the origin of the cultivated species of wheat. The occurrence of species with chromosomes in multiples of 7 might suggest that the 14- and 21-chromosome groups have originated from the primary 7 chromosomes through reduplication by doubling and crossing. Pollen grain size is closely associated with chromosome number, indicating that the larger chromosome numbers are not the result of chromosome fragmentation. If the Vulgare wheats contain 3 sets of chromosomes from the same original source they must have changed considerably because 7 of the Vulgare chromosomes are quite unlike the primary 14. Moreover, with 3 similar sets of chromosomes we should expect assortment of chromosomes in hexasomic sets which with random assortment of the chromosomes would result in genetic ratios of 35 to 1 or 399 to 1 in F_2. Such ratios are not found in wheat hybrids. Even the primary 14 chromosomes of the Vulgare

wheats are probably not homologous because Gaines finds no pairing of chromosomes in the reduction divisions of a haploid plant.

According to Percival the Vulgare wheats may have originated from a cross of *Aegilops* with a wheat of the Emmer group. *Aegilops* and particularly *A. cylindrica* possesses a number of typical Vulgare characters not found in the Emmer wheats, such as rounded keel, hollow stems, susceptibility to rust, and winter habit of growth. The combination of the *Aegilops* characters with those of the Emmer wheats is thought to account for the great diversity of the Vulgare varieties as well as for their specific characters. The cytological data tend to support this view. When *A. cylindrica* is crossed with *Triticum vulgare* there are 7 pairs of chromosomes at the reduction division indicating that the 2 genera have one set of chromosomes in common.

Gaines finds that when *A. ovata* is crossed with an Emmer wheat there are no paired chromosomes at reduction. He suggests that there are 4 sets of chromosomes in the 2 genera, *Aegilops* and *Triticum.* Einkorn has sets A or B, the Emmer group has sets A and B, the Vulgare group has sets A, B, and C, while *A. ovata* has sets C and D. Presumably chromosome set C of the Vulgare wheats came from *Aegilops* while sets A and B were derived from the Emmer wheats.

The recent work of Tschermak and Bleier does not seem to support this view, however. These investigators obtained a homozygous fertile segregate from a cross of *Aegilops ovata* with an Emmer wheat. A cytological examination showed that the F_1 hybrid had 28 pairs of chromosomes due to the doubling of the chromosome number in the first division of the fertilized egg. This new species with 28 chromosomes is quite unlike the wheats of the Vulgare group. However, additional work with crosses of *A. cylindrica* and Emmer wheats may show different results.

The work of Tschermak and Bleier with wheat hybrids, Blackburn and Harrison's work with roses, and Goodspeed and Clausen's results with *Nicotiana* hybrids suggest still further opportunities for cytological research. If methods can be developed to cause doubling of the chromosome number in the fertilized eggs of species crosses, new species can be obtained which might be of economic value. Belling's work suggests that chromosome doubling in the pollen grains may be induced by cold. During the past winter I have attempted to change the chromosome number in cereal hybrids by subjecting them to cold in the greenhouse. Certainly the F_1 hybrids between the Vulgare and Emmer wheats are more vigorous than either parent and if the chromosome number could be doubled so that these hybrids would be homozygous and fertile they would undoubtedly be more productive than the parental species. Extensive experiments along this line would, I believe, be justified.

The cytological investigations with *Triticum* have shown the reason for sterility in species hybrids, uncovered the difficulties to be encountered in cereal improvement through species hybridization, and have indicated the possible origin and genetic relationships of the wheat species. Although all of the problems of species relationships and genetic results have not been solved, cytological methods have made clear many of the difficulties encountered and will undoubtedly be an important factor in further investigations.

MORPHOLOGICAL AND HISTOLOGICAL PECULIARITIES OF THE PODOSTEMONACEAE[1]

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Three years ago I had the opportunity of making observations on living specimens of Podostemonaceae during a stay in our Dutch Colony of Surinam (Dutch Guiana). A small expedition went into the interior as far as the Raleigh Falls of the river Coppenam, where a camp had been prepared beforehand. A steam launch brought us to the last negro village; from thence we proceeded with the help of the bush-negroes in their canoes. These canoes are so built that they can easily pass the rapids.

Due to the care of the leader, Dr. Stahel, the camp was prepared in such an excellent way that we found even a small laboratory, where we could make microscopical observations. Here I remained for about a month, together with my son who acted as my assistant, so that I will give here a short account of our joint work. We were far away from any human settlement, in the center of the immense tropical forest stretching to the Amazon river in Brazil. A bare granitic rock in the neighborhood, the Voltz Mountain, gave us the opportunity of catching a bird's eye view from above, and from this vantage point the eye could discover nothing else but the mighty rain forest. Just below our camp were falls in which Podostemonaceae were growing, though we could not reach these. At a distance of about a quarter of an hour, however, we found a small waterfall, where investigations of the living material could be made on the spot.

In a few words let me remind you that Tulasne gave us a great monograph of this family and that afterwards they were studied more especially by Warming and, as far as the species from India and Ceylon are concerned, by Willis. The last named naturalist did study living forms; in America this has only been done by Goebel, if we exclude the merely incidental observations. And even Goebel could give but a few days to his observations, so that by a longer stay in the neighborhood of their natural habitat, it seemed to me as if it might be possible to add some new facts to those already known about these curious plants.

However, a month is much too short a time in which to get an idea of their mode of life. One should stay at least a year, or longer, in such a spot. For instance, I saw nothing of the way in which germination takes place. On the other hand, these plants produce only stems and leaves as long as they are submerged; flowering occurs when the water in the river has fallen so much

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany, Ithaca, New York, Aug. 17, 1926.

that the leaves are becoming dry. During our stay at the Raleigh Falls the river was rather high for the period of the year on account of heavy rain; and it was only during the last days that we were able to make observations of the flowers. Some weeks afterwards, though, I had the opportunity of seeing the flowering stages in the falls and rapids of the Surinam River near Kabel Station, which can be reached by rail in about 6 hours from Paramaribo.

I shall now try to give you a general survey of my observations. First of all, I shall take a large form, *Oenone Staheliana*, as a representative type. It has long, floating stems with alternate leaves in 2 rows. No growing point of the stem can be detected and the leaves sprout out from each other, every younger leaf being produced by the one next older. In this way the stem is composed of a number of phyllopodia and the whole plant is a beautiful demonstration of the caulome-theory of Celakovsky and others. Each leaf has a base, which is not divided into lobes, whereas the apex generally is more or less, sometime even strongly, branched. The leaf is densely covered with appendages, club shaped, which in reality are ranged in 2 rows, one on either side of the midrib. These have been called "gills" by Goebel, somewhat unnaturally because they have nothing to do with respiration, but are rather assimilatory organs. They are deep green in color, brought about by the great number of chloroplasts in the cells of the epidermis; the same may be said of the lobes of the leaf, which have the same structure. Generally in the Podostemonaceae the chloroplasts are restricted almost entirely to the epidermis, coating the inner cell walls, whereas the outer ones are coated by light green leucoplasts. Very few chloroplasts are to be found in the subepidermal layer of cells, whereas the inner parenchyma is destitute of green color. The absence of stomata in this family is generally known.

A curious fact, which can easily be detected with living plants, is the large number of hairs on these "gills" and on the lobes of the leaves. The hairs are very easily thrown off and so generally are not seen in material brought to the temperate regions. The hairs have a small basal cell and a long apical one, the latter is movable with regard to the basal one. In consequence, one may see these hairs all moving to and fro with the movement of the water almost like ears of rye moved by the wind. It is very probable that these hairs are the principal absorbing organs of the plant; at least in plasmolysing one can see that the walls are extremely permeable to the plasmolysing fluid, much more so than other superficial cells.

Perhaps this is the place to note that generally Podostemonaceae need a great deal of light; when a cataract or rapid is much in shadow, these plants are wanting. Perhaps also in this way may be explained the fact that in the vicinity of the banks of the river no Podostemonaceae are to be found, though the stones on the same spots may be densely overgrown with liverworts or filmy ferns.

Oenone Staheliana lives in the largest falls and consequently the stems are knocked up and down by the waves of the torrent. How is it possible that they resist such a stress? I first thought that perhaps their turgescence might be very high, but such is not the case. By plasmolysing, it was ascertained that the

osmotic value of the cell sap is not much higher than 10 atmospheres. Their mechanical tissue is collenchyma, more especially as a sheath around the vascular bundles, which in themselves show rather a poor development. The phloem though, can be detected easily but the xylem generally must be carefully sought for; in many cases it is only represented by a few annular vessels.

All the shoots are produced from creeping roots. These flattened dorsiventral roots are firmly fastened to the granitic rock in a manner to be described a little later. They produce what may be called adventitious shoots, though the term does not sound very exact here. The shoots arise laterally at the ventral sides of the roots, generally one on each side, breaking through the outer tissues. The roots contain chloroplasts, so that they can assimilate the carbon dioxide. As already mentioned, I did not get germination and hence am not able to say how the first root arises; but they branch freely and certainly these shoots have nothing directly to do with the embryo.

The roots are fastened to the substratum by root hairs, which secrete some sticky substance, by which they adhere to the rock. This can be seen more especially in such cases where a plant is growing on a root or a stem of a climbing plant, hanging in the water; these were seen in more than one species of the Podostemonaceae. Of such material microscopic slides could easily be made and these gave the impression that the roots or the root hairs secreted some substance which could dissolve parts of the wood of the substratum so that it became possible for the hairs to penetrate into the woody parts.

The branching of the shoots may be seen when flowers are formed, but I will pass this over because nothing new can be added to the facts discovered by Warming. Suffice it to say that the flower buds remain on the inside of the shoots, surrounded by the spathella, as long as the shoots are submerged. But once the water of the river falls, the flower stalk stretches and the whole plant is covered with flowers of a beautiful pink color, rarely solitary, but generally in inflorescences. A great mass of reserve material, in the first instance starch, has been stored; so the flowers can develop very rapidly. At the same time the leaves and the non-flowering stems begin to decay and even the peripheral parts of the flower stalks are cast off. Cells of the epidermis and of the cortex loosen and are thrown off in the living state even whilst they are strongly turgescent. In consequence of this phenomenon these stalks give the impression of being covered with grains of sand. Simultaneously the sheath of collenchyma surrounding the vascular bundles is changed into sclerenchyma; hence fruiting plants are very unattractive and offer almost no resemblance to the beautiful green forms submerged in the water of the rapids.

The flowers emit a very delicate fragrance; the pollination is effected by small bees (according to Dr. Friese, *Trigona ziegleri* and *T. clavipes*) collecting pollen. We saw them in great numbers flying from flower to flower, but could never detect them on any other kind of flowering plant. On the other hand we only saw them in our "laboratory" when flowers of *Oenone* were brought there.

An account of the development of the embryo sac and of the egg apparatus will be given later on. But we will now first see how the other Podostemonaceae can be derived from the one just described. First of all there are forms in which the leaves show greater development. This is the case with *Oenone Richardiana*, though this species is smaller. But the leaves are broader and hence more conspicuous in regard to the size of the whole plant.

But we had better speak at once of the forms extreme in this direction, more especially of *Mourera fluviatilis*. Here the stem is so much shortened that we find only root leaves. These have an enormous size; I saw some of them of a length of 3 meters. They are beset with a large number of warts, also with some thorny excrescences. The warts have the same function here as the "gills" on the leaves of *Oenone Staheliana;* the color is dark green, whereas the other surface of the leaf has only a very light green color. I have tried Sachs' Iodine experiment, by covering a leaf with a tin plate out of which letters had been cut. At the end of a day the leaf was submitted to the starch test, with the result that the parts which had been exposed to the light became dark blue, whereas the other parts showed only a few traces of starch, with the exception of the nerves, where the parenchyma carried some starch. Hairs are abundant, even more so than on *Oenone Staheliana;* it is rather a curious sight to find these plants, hairy to an extreme degree, growing in the water. An enormous amount of food-stuff is stored in the base of the stem and in the young inflorescence; consequently this latter can grow out in a very short period. I will not discuss these inflorescences, since they have been described several times already, and I have nothing new to add to these descriptions. I should like only to remark that the appearance of the flowering plants in the rapids is fascinating, but so strange that there will scarcely be found a formation on earth which can be compared with it. Here again the leaves decay rapidly and in the fruiting condition the plants with their stiff brown stalks without leaves offer no longer any attraction to the eye.

The plants of *Mourera fluviatilis* are found separate one from another, fastened with rhizoids projecting from the surface of the stems, or from the haptera, the latter curiously twisted organs, of which the morphological value is unknown. Sometimes I thought them to be metamorphoses of roots, but at present I do not hold this opinion, because they are certainly exogenous, whereas roots in Podostemonaceae are endogenous, just as with all other plants. The haptera are very sensitive to contact, behaving like tendrils, and we might perhaps compare them to these, only with the difference that they develop in water.

I found no roots on *Mourera fluviatilis*, the plants sitting, as it were separately on the rocks. Now, it might be supposed that these plants had developed as adventitious shoots out of creeping roots, these having decayed afterwards. But this supposition did not seem very probable and indeed became incredible after the examination of very young plants. I found several young plants with only very few leaves; from their forms I inferred that they really were primordial leaves, more especially by the thin appendices of the limb. At first I had the impression that creeping roots were present; but a closer investigation showed

that several species of Podostemonaceae were growing together and that all the roots belonged to another species, not of the genus *Mourera*. I did not see germination, but leaving this out of the case it may be said that roots are wanting in *Mourera fluviatilis*.

Another direction in which Podostemonaceae have developed is rather the reverse of the case of *Mourera*. Here the roots become very conspicuous and on the other hand the shoots are little developed. These smaller forms may be found in great number also in tropical America, though they are more numerous in Africa and still better represented in India and Ceylon.

Let us take for example *Apinagia perpusilla*, with creeping green roots, producing shoots, generally in pairs. These shoots remain small, and in so far differ from those of *Oenone Staheliana*, as the phyllopodia remain short and join in a kind of thallus, reminding one of Hepatics or other lower plants. The leaves have a basal part which shows no incisions, whereas the apex has thin, gill-like lobes; also here hairs abound.

The small flowers develop very rapidly. A piece of rock densely covered with these plants in which the flower-buds were yet closed, was taken out of the water and deposited in the laboratory at 11 o'clock in the morning. Directly, the flower stalk began to stretch so speedily that at 4 o'clock of the same day the flowers had already protruded from the spathella, so that on the next norning at 6 o'clock the plants were flowering profusely, and the same bees were collecting pollen that pollinated *Oenone Staheliana*.

This species was growing at a greater depth than *Oenone Staheliana* and *Mourera fluviatilis;* about in the middle depth between these I found *Oenone Richardiana*, which I have mentioned already. Now the curious thing was that in the Surinam River where I collected afterwards, in September, 1923, *Mourera fluviatilis* is very abundant, but *Oenone Staheliana* is absolutely wanting. At a greater depth we did not find *Oenone Richardiana*, but a somewhat similar species, formerly described as *Oenone marowynensis;* while still deeper, instead of *Apinagia perpusilla*, two other species of *Apinagia* were found growing together, *A. flabellifera* and *A. nana*. One might speak of vicarious species in these two rivers, the Coppenam and the Surinam.

Apinagia flabellifera seems to me to be identical with *Oserya flabellifera*, and I think it unnecessary to retain this genus of Tulasne. The shoot is even more reduced than in *Apinagia perpusilla* and the greater part of it is pressed against the rock so that the roots with the stems seem to form one simple thalloid mass. In a higher degree the same may be said of *Apinagia nana*, where also the flowers scarcely protrude out of this very little differentiated thalloid mass. But in reality the development is not different from that of the larger forms.

Sometimes the alternate position of the leaves is very distinct, in other cases the development may be traced in examining young roots.

The experiment already discribed for *Apinagia perpusilla* was repeated with *A. nana* and *A. flabellifera*. Pieces of rock were taken out of the river and deposited on a dry spot. In the course of the same day the flowers began to open, whereas beforehand only flower buds could be detected; on the next day, even,

some ripe fruits were found. It is evident that the whole development of the seed goes on with extreme rapidity, during the short period when the water of the river falls so much that the plants become dry. They would not stand this very long, so in a few days the whole generative process comes to an end.

Now, if we look at the Asiatic forms, it is evident that the roots there become even more flattened and thalloid, taking over the function of the leaves, whereas the shoots are extremely reduced. The African forms stand about intermediate between the American and the Indian forms. I have not investigated these latter ones personally, but we know every detail of their life history through the beautiful work of Willis. I myself have only seen the species which till now is the most eastern one known, that is, *Cladopus Nymani* from Java, a species so small that it might easily be overlooked. This makes it possible that one or more of these extremely minute species occur even farther eastward, though perhaps this is not very probable.

We might suppose that the American Podostemonaceae, such as *Oenone Staheliana*, were the most primitive, and that from these two lines development proceeded; one leading to the rootless *Mourera fluviatilis* in tropical America, the other one through Africa to India, Ceylon, and at last Java, giving such extreme forms as *Cladopus*. In *Cladopus* the root is the principal part of the plant, at least the only assimilating one, whereas the shoots are very much reduced. We might even go one step farther and say that this distribution must have begun during the time when, according to the theory of Wegener, America, Africa, and India were one continuous continent, before they separated. But I rather think that it is not very safe to go on speculating in these lines, however attractive they may seem to be. We like to stand on the firm basis of fact and not drift away on hypotheses, where the foundation is wanting.

And we should consider that not only do we know nothing about fossil Podostemonaceae but up to the present we have not been able to grow one single species artificially. So long as we cannot do this, we know nothing about the range of variability of the several forms, or of the influence of environment. It might be, though it does not look probable, that nearly related species were only local varieties, influenced by the surroundings. On the other hand I got the impression that hybrids do exist between several species and that these split up into different forms. But so long as cultivation is impossible, all these questions will remain unanswerable. In my opinion, the first thing requisite is that some botanist locate for at least a year on the spot where many species grow, in order to study their development. One of the cataracts of Surinam would be a very suitable place for this.

Many years ago I studied the development of the embryo sac and the egg apparatus in some of the Podostemonaceae. I found this to be very different from the great majority of the dicotyls; moreover, all species of this family behaved in exactly the same manner in this respect. I was able to confirm this with a complete series of stages of species hitherto only incompletely known. Let me take as an instance *Apinagia perpusilla*. Here the nucleus of the megaspore mother cell divides heterotypically and two cells are formed, of which the upper

one becomes abortive either directly, or sometimes after another division in a plane perpendicular to the former division. The inferior nucleus divides again, and this is not followed by a division of the protoplasm of the cell. The chalazal of these 2 nuclei disintegrates, the other one divides into 2, which shortly afterwards show another division in directions perpendicular to one another. Then the protoplasm divides, giving rise to 2 synergids, 1 egg cell and a very minute embryo sac with 1 polar nucleus. This development goes on in the small apical part of the nucellus projecting out of the inner integument and only covered by the outer one. On the other hand, the remaining part of the nucellus, which is surrounded by the inner integument, cannot follow the development of this latter organ. This part is then drawn out into long strings, which fuse into a large cavity having the appearance of an embryo sac, and into which the embryo grows after fructification. No trace of endosperm is found.

After the appearance of my former article on the embryo sac and the egg apparatus Werner Magnus from Berlin studied several species from India and Ceylon in this respect and though, generally speaking, he got the same results, yet there were some differences in detail. It was not impossible that we were dealing with a difference between the American and the Asiatic species, and so I thought it necessary that an investigation should be made of *Cladopus Nymani*, the species from Java. This was carried on by one of my students, Miss Frida Beucker Andreae, who, however, became seriously ill during the work and I regret to say, that, before she could finish it, death took her away. But she left some manuscript and many preparations, which made it possible to give an account of her work. I will not repeat this in detail, more especially because the result indicated perfect agreement with the American forms. So here again no clue could be obtained as to the origin of the Asiatic forms.

There is 1 other minute species about which I should like to say a few words because it belongs to a suborder, which Willis even wants to separate from the true Podostemonaceae. I mean *Tristicha hypnoides*, which I studied in the Coppenam and in the Surinam River. It has 3 rows of leaves, but also here the transition from leaf to stem is gradual, so that there is no line of demarcation even microscopically. The shoots here also arise from roots and there is generally so much in common between these plants and the other Podostemonaceae that the structure of the flower seems to me insufficient as a basis of separation; also the development of the embryo sac and the egg apparatus is the same, as far as I could see. Now *Tristicha* is American and African, whereas the other genus *Lawia* is Asiatic, so the distribution here again gives no clue as to the way in which development has taken place.

Before finishing, I wish to draw your attention to the milky sap, which extrudes from *Mourera fluviatilis*, when a cut is made. An investigation showed that this fluid is contained in long tubes with many nuclei; every one of these tubes develops by the growth of 1 single cell. It soon became evident that the same kind of latex is to be found in the other species, though the laticiferous cells in these cases are not so long, even may be rather small, as for example, in *Tristicha hypnoides*. This made it possible to study the contents of these lati-

ciferous cells under the microscope without damaging them, and in this way I determined especially by plasmolysing, that the small particles in the latex are in reality inclusions of the cell-sap.

Thus the observed facts lead us to the same conclusion arrived at by Molisch and lately Bobilioff, that is, the latex is a constituent of the cell sap, and is to be found in the interior of the vacuole. This seems to be true even when this latex is different in some respects from that in *Hevea* and other plants. But who can say what are the real characteristics of latex?

THE EMBRYOGENY OF THE CONIFERS

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In this discussion of the embryogeny of conifers, I shall not confine myself to a review of certain former publications, but shall include the results of some later studies which have not been published. The new material includes *Biota orientalis* (usually but erroneously merged with *Thuja* in our manuals), *Libocedrus decurrens* and a few observations on *Thuja occidentalis*, as well as the results of a study of certain podocarps: *Dacrydium*, several species of *Podocarpus*, and *Phyllocladus*. For this material I am greatly indebted to Dr. E. W. Sinnott who placed an extensive portion of his New Zealand podocarp material at my disposal, to Dr. W. C. Coker who furnished me with a number of ovules remaining from his investigations ('02), and to Dr. A. J. Eames for material of *Agathis*. This alcoholic material was dissected and studied by methods which I have used in the past on other conifers. I am also indebted to Dr. L. L. Burlingame who supplied me with living cones of *Libocedrus decurrens* in cultivation on the Stanford University Campus. The *Biota* material was obtained from cultivation at Canyon, Texas.

The embryogeny of conifers represents a greatly neglected though important phase of morphology. The great diversity in the manner of embryo development in this group of plants might seem to indicate, at first sight, that the embryogeny of conifers is a highly variable process, one which would, therefore, be of very little assistance in the solution of the problems of phylogeny. The variation in the number of cotyledons in pine embryos of a single species and the variation from species to species would be enough at the start to discourage anyone who might wish to undertake a study of the embryogeny. However, we shall consider for the most part, the early stages following the organization of the proembryo, those stages which come between the proembryo and the organization of the embryo into tissue regions and organs. These stages are the ones in which I have examined a large series of the various conifers by means of dissected preparations and can base my knowledge very extensively on my personal investigation. In these early stages of the embryogeny of conifers there are also many diverse features. Many of these features, as I shall attempt to point out, are variations due to the complications of cleavage polyembryony, but with the interpretation which I have proposed and developed ('18, '26) by which the embryos of all conifers may be regarded as made up of several or many distinct units or parts

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often combined into one, many of these apparent diversities become significant as contributing to the unity of the evolution of embryonic structure.

While the early embryogenies of the various genera are even more diversified and complex than was suspected, they are not found to be highly variable at this stage in any single form; in fact, they are usually very nearly constant in essentials for a genus. In some groups the type of embryogeny is fairly constant for sub-families. So it happens, as I will attempt to show, that the diversity of embryogeny found in conifers as a whole shows much fundamental unity, and that embryogeny is a very useful means of discovering certain relationships and differences among the groups of genera and families.

Embryogeny is also useful in corroborating taxonomic distinctions between genera. For example, we find that while *Pinus* and *Cedrus* are essentially very similar, *Tsuga* and *Pseudotsuga*, merged by some systematists, are much more distantly related according to the testimony of their embryogeny (Buchholz '20). Similarly, as I shall presently point out, *Biota* and *Thuja*, which are usually classed together as *Thuja*, are dissimilar in their embryogeny. *Biota* and *Libocedrus* are very closely related to each other and to *Juniperus communis*, as we may infer from Strasburger's account and from the figures of the latter ('72) while *Thuja* is a distinct and more distant derivative.

One of the difficulties which has hindered progress in the study of the embryogeny of the conifers has been that of technique. The general use and absolute dependence upon the microtome has probably been a factor which retarded progress in the successful study of conifer embryogeny in recent times. Strasburger and his contemporaries who were active in this field were largely dependent on free hand sections or dissected preparations. Some very excellent studies have been made recently with microtome methods, especially in the case of araucarian embryogeny (Eames '13, Burlingame '15), but in most other conifers I find from my own experience that the best results are obtained by methods of dissection; by teasing out the embryos from the ovules, in the living condition if possible.

Of course, serial sections must also be used. They are indispensable for a study of fertilization and the proembryo stages, but the stages of suspensor development and elongation are not easily understood from a study of the sections alone. The many twists and coils of the suspensor cells, especially when they have become fully elongated or have begun to collapse, cannot be followed and reconstructed from serial sections; these must be studied from dissected material.

In the later stages of embryogeny, as the embryo becomes more massive and multicellular, the cell structure may be studied advantageously by microtome sections, but even here a study of dissections affords a valuable check on any conclusions concerning the manner of suspensor development.

It appears from some preliminary work which I have done that this later stage of the embryogeny when the tissue regions are organized and differentiated is the stage in which the greatest general uniformity may be found in the embryogeny of all conifers. However, I am not including these stages of the

embryogeny in this discussion, largely because too little is known about them at the present time.

In order to give an idea of the procedure in making dissections, I will illustrate this method by a brief outline of this technique for *Biota*, a form in which I have been interested recently.

Technique

Dissections should be made from the living material under water or preferably under a 0.3 gm. molecular sugar solution. The gametophytes (prothallia) are removed from the ovules and placed in the sugar solution in a watch glass or Petri dish and the dissecting is done under a Greenough binocular dissecting microscope, utilizing both transmitted and reflected light.

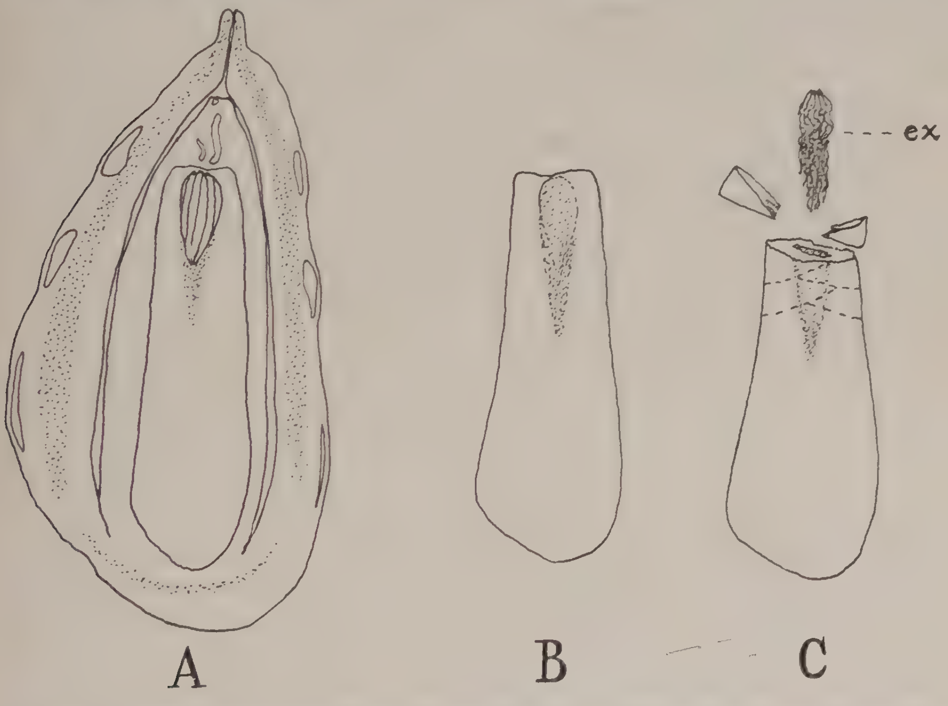

Fig. 1. Dissection method for *Biota* (*Thuja orientalis*). A. Section through an ovule near the time of fertilization showing seed coats, etc. with pollen tubes in nucellus, and archegonial group in upper region of prothallium. ×28. B. Prothallium of slightly later stage removed from seed coats, showing area with archegonia and cavity containing embryos as observable by transmitted light. C. Embryo complex (*ex*) removed from cavity within prothallium, showing method of cutting off wedge-shaped portions in the dissection.

An ovule of *Biota* when cut in longitudinal section about the time of fertilization appears as A, Figure 1. A gametophyte after removal from the testa has the appearance of B, Figure 1, and the archegonial group may be recognized by the transmitted light near the smaller end. If the embryos have developed to the

stage in which suspensors have elongated, this funnel shaped cavity in which they lie may be recognized by the difference in the translucency of the tissue below the archegonia, the area shaded in B.

A pair of fine-pointed forceps with weak springs should be used in one hand to grasp the gametophytes and a double spear point needle in the other for the dissection. The tissue surrounding the archegonia at the upper end is removed first. Then a little teasing and cutting away around the margin will usually loosen the archegonia on all sides. If the embryo complex does not protrude with this operation small triangular pieces (C, Fig. 1) may be cut and broken off alternately from opposite sides, turning the ovule over after each incision, until the embryo complex becomes loosened and floats out, shown at *ex* in C, Figure 1. It should be transferred immediately by means of a pipette to a killing solution and for this we have found formalin-alcohol (6 per cent formalin in 50 per cent alcohol) very satisfactory. They may be left in this killing solution for an indefinite period—until a convenient time for staining.

Preserved alcoholic material may be dissected if it has been well fixed, but is far less satisfactory than the living material, and the embryos must be handled much more carefully. Very often ovules killed and fixed by the ordinary routine technique used in imbedding show the suspensor portions of the embryo collapsed or partly digested. I believe this is due to the action of the enzymes secreted by these structures. After the killing of the parts, the enzymes which have accumulated will sometimes attack the delicate walls of suspensor cells and corrode them beyond recognition.

Staining may be done with Delafield's haematoxylin. I always wash out the killing-preserving fluid in water, and place the embryos in the stain for 5–10 minutes, which usually overstains them. Then I de-stain under a microscope to the desired point (when the suspensor cells are still tinted) by using very weak acid (one drop HCl to 50 cc. water), washing thoroughly afterwards (sometimes neutralizing with a trace of ammonia or lime in the water) and place them in 10 per cent glycerin for dehydration. The latter method eliminates unnecessary handling, and may be accomplished rapidly if the mass of embryos is placed in a group in about 1–2 cc. of 10 per cent glycerin on a glass plate. Here the water evaporates rapidly leaving the embryos in concentrated glycerin in a few hours.

They are washed out in 95 per cent alcohol, followed by absolute alcohol and put into 10 per cent Venetian turpentine in a dessicator and then mounted in this medium on slides, when the consistency has reached that of thick glycerin.

More recently I have found another method, avoiding the use of Venetian turpentine. From the absolute alcohol, I place the embryos through a series of 25 and 50 per cent, and pure Diaphane (a new mounting medium soluble in alcohol and furnished by the Will Corporation), in which they are mounted. If alcoholic material is dissected and it is desirable to avoid the handling incident to an aqueous stain, magdala red may be used in the 95 per cent alcohol to which they may be transferred after dissection from 70 or 85 per cent used as preservative, a method which I have used with some success on alcoholic podocarp

embryos. The methods for *Biota* as well as other conifers are very similar to those which I have described for *Pinus* ('18).

Suspensor Structures

One distinctive structure which all conifer embryos possess is a remarkably developed suspensor system (Fig. 10). These structures are shown to good advantage by means of dissections. With the archegonia usually placed at the ends (or occasionally at the sides) of the gametophyte, some special organ must be provided which can place or push the embryonic cells more deeply into the prothallial tissue. Suspensors which perform such a function are found in some ferns and according to Land ('23), even some Bryophytes have an organ which may be considered a suspensor. Land looks upon these primitive suspensors as either haustorial structures or organs of thrust, pointing out that (among these lower archegoniates) they develop in forms in which the embryo is excentric to the principal food supply and are usually absent when the embryo is concentric to the food supply.

But among conifers the suspensors are much more highly specialized. They are more than mere haustorial organs. It is possible that the suspensors are also secreting organs, and as organs of thrust, they are the weapons by which the embryos struggle in a process of embryonic competition. The embryo which finally succeeds in the competition against its fellows and comes to remain as the surviving seed germ has gained this advantage by demonstrating greater growth vigor; by having proven its capacity to produce a stronger and stiffer suspensor than its fellow adversaries. This internal process of embryonic selection has been operative for a long period of time, so that, through selection, suspensors have become greatly over-developed structures, usually many times as long as would be necessary if the suspensor were merely an organ which moves the embryonic cells into the center of the region of the food supply.

It is highly probable that the suspensor may also be a secreting organ. We learn from animal histologists that secreting cells such as those in the digestive tract are columnar elongated cells with large vacuoles. This description fits all suspensor cells. The elongating suspensor cells may be the cells that supply the enzymes which break down the tissue in the center of the gametophyte. This function must be performed either by the embryonic cells themselves or by these suspensor cells and the embryonal tubes which elongate from them. That this is a function not always possible on the part of the embryo-forming cells themselves is shown in several examples of conifer embryos. This condition is illustrated by the cases of *Araucaria* and *Agathis*, in which the embryonic cells which ultimately contribute the embryo are completely surrounded and enclosed by elongated or otherwise enlarged cells, leaving no exposed surface for the outlet of any fluids which they might excrete. Yet during this period while the embryonic cells are completely surrounded by suspensors and cap, the cavity in the prothallium is formed. Another instance of this kind may be taken from the embryos of *Biota* and *Libocedrus*, both of which develop very thick inner walls (Fig. 11) immediately after cutting off their secondary suspensor cells. These embryo cells remain

for a time inactive and retain a dense protoplasm, which would indicate that they are not active in the secretion of enzymes. However, during this time, the suspensors become long and clear with large vacuoles, and the prothallial cavity enlarges correspondingly, indicating that the activities of secretion are going on as well as the obvious mechanical work of the suspensors in keeping their embryos pushed forward as far as possible. Other instances of this kind might be cited. Thus, there is some good evidence to indicate that the suspensors, rather than the embryonic cells, are the secreting organs.

However, the suspensor of the successful embryo must remain relatively turgid and firm, under the condition of struggle between the embryos. The embryo must become multicellular as rapidly as possible, for multicellular embryos give off stiff massive suspensors made up of many embryonal tubes. The divergent embryonal tubes often serve as hooks or spurs by which the smaller embryos which remain behind are pushed back to the archegonial region of the cavity within the prothallium (Fig. 12, larger embryo below, at left).

If the enzymatic action of the suspensor cells constitutes one of their functions, then this is an activity which benefits the entire community of embryos within a gametophyte. It is subject to localization only after the successful embryo has separated itself sufficiently from its neighbors to render it dependent on the local action of its own suspensor cells. However, the mechanical thrust of the suspensor is an individual quality which bestows its benefits directly on the embryo contributing the suspensor. Embryonic selection, therefore, favors stiff, well-developed suspensors. Enzymatic secretion is probably more in the nature of a function which has become secondary, but goes hand in hand with the processes of suspensor growth.

Simple Polyembryony and Cleavage Polyembryony

When several embryos are found, each one a different zygote, derived from a different egg, the polyembryony which results is called simple polyembryony. Here the polyembryony is due only to the existence of several archegonia. The spruce (*Picea*), larch (*Larix*), Douglas fir (*Pseudotsuga*) and American arbor vitae (*Thuja occidentalis*) afford good examples of simple polyembryony. When the zygotes each give rise to several embryos the condition is spoken of as cleavage polyembryony. This form of polyembryony is found in the pine (*Pinus*), hemlock (*Tsuga*), Oriental arbor vitae (*Biota orientalis*) and Incense cedar (*Libocedrus*). The embryos coming by cleavage from one zygote constitute a system of embryos; when several embryo systems are intermingled and confused, the embryonic mass may be spoken of as an embryo complex.

Cleavage polyembryony was no doubt derived originally from the condition of simple polyembryony, for no instance is known among Pteridophytes where the zygote gives rise to several embryos. During the transition from ferns to seed plants or very soon after this time of transition, cleavage polyembryony had its origin.

That the origin of cleavage polyembryony was not a recent event in conifers may be inferred by a more careful consideration of the process of embryonic selection.

Embryonic Selection

Embryonic selection is a special form of developmental selection (Buchholz '22) in which many embryos struggle for supremacy. In this struggle, the embryo possessing the greatest growth activity represented by a stiff suspensor soon gains an advantage, usually indicated by its foremost position with respect to its competitors (Fig. 10). It eliminates all of its fellows sooner or later, for practically always, only one embryo remains in the mature seed. The elimination usually takes place in the earlier stages of development, so that by the time the largest embryo has begun to organize its cotyledons, there remain only very small traces of the checked embryos. Frequently the successful embryo may be recognized at an early stage, as the one foremost in position. In this elimination, chance plays a minor rôle, for the persisting embryo usually merits this reward. This conclusion is based upon observations of an extensive series of embryos of different conifers, dissected out in various stages of development. It seems clear from such studies that the embryo possessing the greatest growth vigor usually wins in the competition, and the suspensor is in reality an organ of competition. The values measured by embryonic selection, expressed mainly as variations in their growth rate, represent differences which are inherent among the several embryos.

In conifers, the processes of developmental selection often measure more than the differences between the embryos, for it really consists of a relay race (Buchholz '22). The successful embryo plus the pollen tube which produced it must be superior to the sum of the pollen tube and embryo of its neighbors within the same ovule. Where cleavage polyembryony is involved, the competition measures the best pollen tube plus the best fraction of its zygote, against many other similar combinations within the same developing seed.

Direction of Evolution of Cleavage Polyembryony

Some light is thrown on the nature of cleavage polyembryony by considering the direction in which its evolution must necessarily proceed. Let us first consider the possible outcome if a plant having simple polyembryony should give rise to the condition of cleavage polyembryony. Could cleavage polyembryony originate and survive under conditions as they exist within an ovule, as it must be assumed that it has in relatively recent time, if cleavage polyembryony is a feature of recent origin?

Suppose, for example, that in Figure 2, representing a condition similar to *Pinus*, cleavage polyembryony does not exist. Let B represent, in this instance, a condition in which the entire zygote forms the embryo, and in which the competition which exists is between B and A, or other neighboring zygotes. Let A represent a departure from the usual condition, a change, such as a mutation, in which cleavage of the zygote is taking place. Under these conditions, what chance is there that any of the several embryos at A might survive? Judging by the observed facts about the competition among conifer embryos, the answer is not difficult; there is little or no chance that any of the smaller embryos at A

might survive. Granted that fertilization of the 2 eggs was simultaneous, and other conditions except cleavage polyembryony the same, since embryo B is so much larger than A to begin with, and since it starts its forward growth with a 4-celled and much more rigid suspensor (in fact it has in the number of its cells a 4-fold advantage over the embryos at A), there can be no doubt of its supremacy from the beginning. Embryo B will probably contribute the successful embryo to the seed, and the change to cleavage polyembryony in A will leave no progeny.

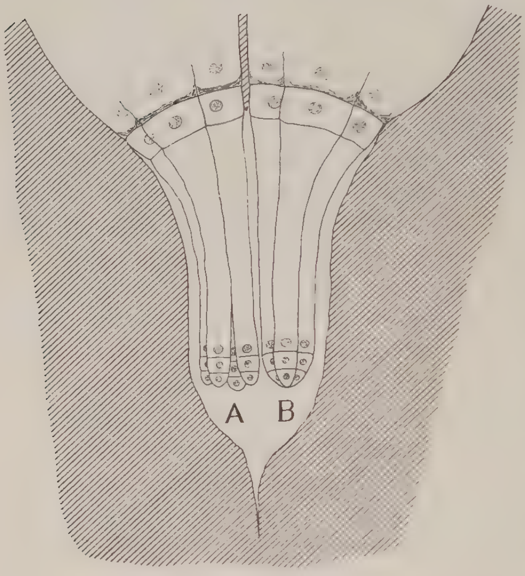

Fig. 2. Two conditions in embryogeny of Abietineae (explanation in text).

Suppose, on the other hand, that in Figure 2, A represents the usual condition, that to begin with, cleavage polyembryony is present as shown in A, and that a mutational change from the condition in A to that in B has taken place. This the time the change will survive, for the new condition B is the larger embryo, equipped with the stiffer suspensor, while the several units of A will lose out in the competition with B. Thus the new condition represented by B would survive the embryonic competition to produce the seed germ, and certainly it would leave a progeny, which could continue and establish this characteristic of simple polyembryony, although in its derivation it came from a condition of cleavage polyembryony. It is therefore not likely that cleavage polyembryony has had its genesis in relatively recent times, but rather that this condition had its origin possibly as early as the time of the origin of seeds themselves. At least, it must have appeared before the mechanism of developmental selection reached its present adjustment; before the combined activities of pollen tubes plus their respective whole embryos from separate zygotes were pitted against each other in the decisive internal competition which exists within the ovules of conifers. It seems clear that embryonic selection would permit evolution to proceed from cleavage polyembryony to simple polyembryony, but not in the reverse direction.

The evolution of simple polyembryony from cleavage may be considered a kind of orthogenesis, but one which can be explained on a mechanical basis; it is a question of embryonic selection. A very definite product of this orthogenetic mechanism is a very large suspensor, which is certainly an over-developed structure when we consider the structure and function of suspensors generally in other seed plants, where embryonic selection is not usually found.

Failure of Simultaneous Fertilization

It follows from the nature of this developmental selection, that the origin of cleavage polyembryony is to be sought under conditions in which only a single egg was fertilized, or in which the fertilization of several eggs was not simultaneous nor nearly so, with the result that the embryonic selection did not eliminate the smaller products of cleavage. This condition of successive rather than simultaneous fertilization would protect the much smaller and weaker embryos coming from the cleavage of an early zygote.

Transition to Pollen Tubes

It would seem probable that during the earliest stages of pollen tubes, when these male gametophytes were, for example, in a state of transition from that of heterosporous ferns to the condition found in pine, these pollen tubes were not able to grow equally well in the sporophyte tissue. With free swimming sperms, as occurring in ferns, simultaneous fertilization of several eggs was the usual condition which initiated the process of embryonic selection, but with the change to pollen tubes, one would expect that simultaneous fertilization of 2 or more eggs would be only rare and very accidental.

The time of fertilization of neighboring archegonia probably varied by weeks or months, where this varies today in living forms by only days or hours. This interval of difference in growth rate of several relatively imperfect pollen tubes was the occasion for the survival of cleavage polyembryony, should it have originated as a changed condition such as a mutation.

Cleavage Polyembryony not Recent

Considering the foregoing, it is difficult to explain how cleavage polyembryony could be a feature of recent origin. Orthogenesis, if we may be permitted to use this term in designating the mechanical tendency to pass into simple polyembryony, favors the elimination of cleavage polyembryony wherever pollen tube competition is sufficiently effective. Cleavage polyembryony was therefore only an incidental event, accompanying or closely following the advent of siphonogamy, and this is why many conifers have returned to simple polyembryony, especially in forms where the pollen tubes afford a competitive mechanism of a sufficient degree of perfection, so that the fertilization of several eggs by competing pollen tubes is practically simultaneous.

Embryonic Changes from Ferns to Conifers

If we wish to picture a hypothetical transition of embryogeny from ferns to conifers we will select a fern of the eusporangiate type as a starting point. A number of living ferns have been reported with suspensors. Whether we must go back to the filicinean line to find the predecessor of conifers or to the lycopod line (which is less probable) we have in either case instances among living forms where the embryo has a suspensor of one or more cells which elongates and pushes an embryonic cell deep into the gametophyte tissue. Such an embryo of

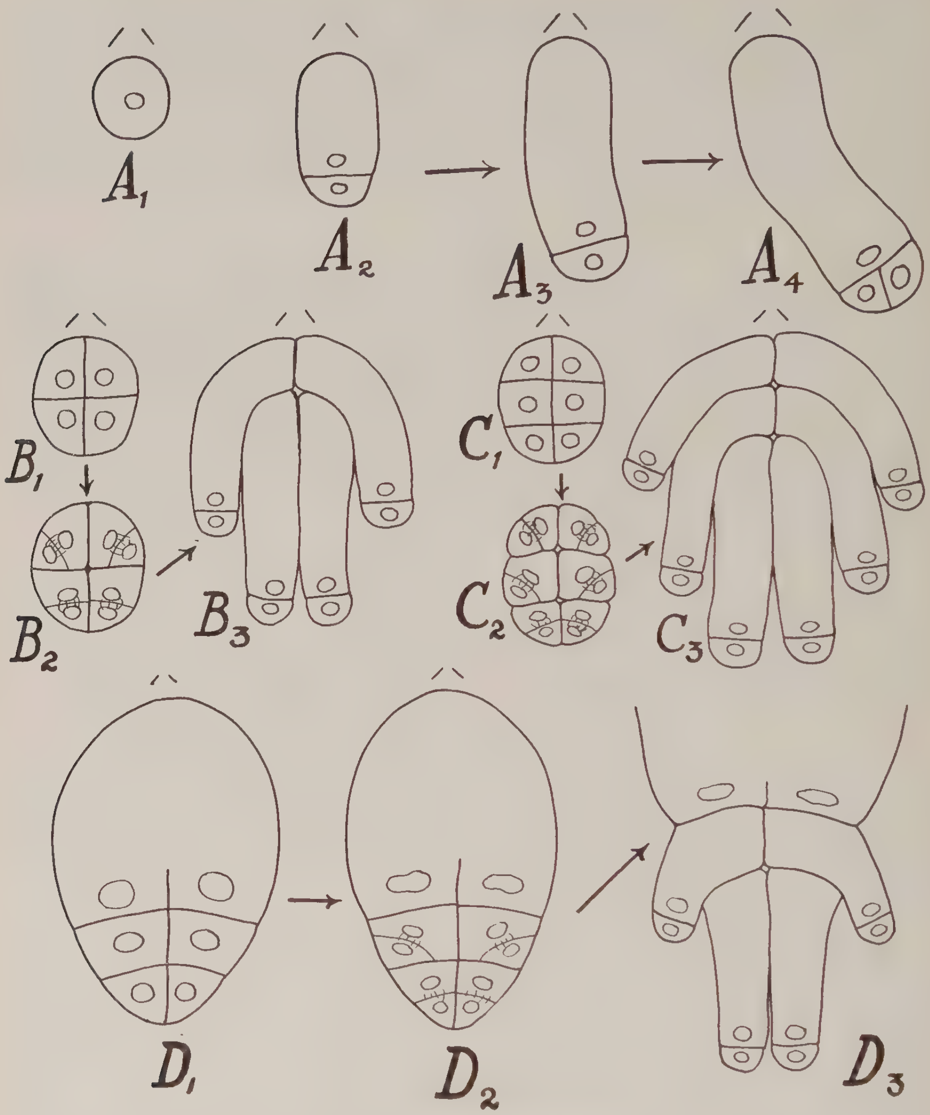

Fig. 3. Diagrams showing possible theoretical steps involved in the transition from ferns to conifers with the introduction of cleavage polyembryony. A. Actual steps in development of early embryo in certain living eusporangiate ferns. B. Hypothetical representation of cleavage of egg into 8 embryo initials (only 4 are shown) followed by independent organization of a single embryo from each initial, and suspensor elongation. C. Same condition, with preliminary cleavage into 12 embryo initials. D_1 condition of C_1 accompanied by an enlargement of egg; D_2, D_3 development of embryos from lowest tiers in D_1 with abortion of upper incompletely walled tier. D should also be compared with stage 5, Figure 4 and stages 6–8, Figure 5.

2 cells is diagrammatically represented in A_2, Figure 3, and resembles in its essentials the embryo of *Danaea* (Campbell '11), *Angiopterus* (Land '23), or even some of the lycopods. With the introduction of cleavage polyembryony in which 8 embryos are formed from an egg, the sections of this embryo system would come to appear as in B_3, Figure 3. Here the zygote has divided into 8 cells, 4 of which are shown, and each of these represents an embryo initial cell. B_1 represents the first step, and in the next step (B_2) each of these cells would divide to form an embryonal cell and a suspensor. After the suspensor elongate, an embryo system resembling B_3 would result—considering in each case a section showing only 4 of the 8 units. With a preliminary cleavage into 12 or more embryo initials the section might appear as in C_1, Figure 3, and this would result in an embryo system shown in C_3, Figure 3. With a pronounced enlargement of the egg and a similar uniform number of cells in each tier (with abortion of the upper tier), the embryo system would appear as in D_1 Figure 3 and the steps following this. An irregularity in the arrangement of tiers would result in the condition of *Biota*, described further on in Figures 6 and 9.

In D_3, Figure 3 we have reached the essential embryo organization of *Pinus* as well as of some other conifers, and the main feature which has been added to the embryogeny of a fern is the cleavage of the zygote into 8 or more embryo initials accompanied by a pronounced enlargement of the egg.

Thus we see that the transition from ferns to conifers such as the pine is not a difficult one on a theoretical basis. Of course this transition may actually have been much more complicated; it hardly seems possible for this change to have occurred on a more simple basis than that which is here represented, except that the number of embryos first formed by cleavage might have been smaller; it may have begun with cleavage into 2 or 4 cells and become gradually more complicated with many embryo initial cells.

Proembryo Stages in Conifers

The proembryo stages of conifers, as this term has generally been used, include the free nuclear divisions of the zygote up to the time when the suspensor cells begin to elongate. The first cell divisions of the zygote usually result in a number of free nuclei which vary from 4 or 8 to 32 or more. Sooner or later these free nuclei become organized with walls in the lower end of the egg (away from the nucellus and archegonial neck) and elongation takes place as soon as the suspensor cells have been formed.

The first stages of the proembryo, the manner of division of the nuclei of the zygote, etc., are important comparable features of the embryogeny, but these are not as well known as the later stages in many cases and I will confine this discussion largely to the stages following the organization of the proembryo. Of course, we are interested in the proembryo, at least to the extent of knowing the origin of its various cells, and in these stages much investigative work remains to be done.

For example, in *Pinus* each of the 8 embryos which comes from 1 zygote may be traced back to a free nuclear division of the fertilized egg, and this may also be done in *Ephedra* (Strasburger '72, Land '07). See stages 1–5 in Figure 4. The critical proembryo stage in *Pinus*, where it is made up of a group of unicellular embryo initials is the 12-celled stage (stage 5, Fig. 4), the stage in which there are 8 walled cells arranged in 2 tiers, and an open tier of free nuclei above these. In the 16-celled stage (stage 6, Fig. 5), which is followed by suspensor elongation, each cell of the lowest tier is related to the suspensor cell above it as part of a single unit; these groups of 2 superposed cells really constitute 4 2-celled embryos. The primary suspensor cells *s* come from the embryonal cell *a* in the same manner as the succeeding secondary suspensor cells $e_1e_2e_3$ etc. In the literature, these secondary suspensor cells are usually referred to as embryonal tubes.

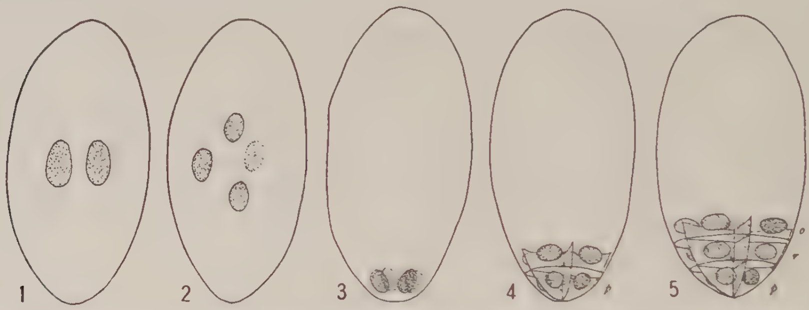

Fig. 4. Five steps in development of proembryo in *Pinus;* diagrammatic reconstructions from serial sections and published figures: *p*, tier of primary embryo initial cells; *r*, tier of rosette cells, nitial cells of rosette embryos; *o*, upper open tier of cells; normally tiers *r* and *o* come from division free nuclear) of upper tier of stage 4.

While in *Pinus* the primary suspensor has the same origin as the later embryonal tubes, structures which appear similar to the primary suspensor cells of other forms such as *Biota*, *Libocedrus*, *Cephalotaxus*, and the podocarps have an origin identical with embryo-initial cells. They are some of the first cells of the proembryo which receive walls, and represent entire embryo cleavage units which were diverted to form suspensor cells. I am introducing the name pro-suspensors to designate these suspensor cells which seem to represent entire embryo initials diverted to the formation of suspensors. Such pro-suspensors also appear in *Pinus* (Buchholz '18) in rare instances.

Pinus and Abietineae

The classification of families and sub-families, and the general order of treatment which I am following in the arrangement of this material, is that used by Coulter and Chamberlain ('10). As the suspensor cells (s) in *Pinus* elongate (stage 6, Fig. 5), the embryo cells (*a*) are pushed forward and each of these four cells divides to form the cells of the first embryonal tubes e_1e_2. By elongation, these embryonal tubes later add to the suspensor. The terminal cells of the

embryo continue to divide by apical growth and all subsequent additions to the suspensors of the respective embryos arise from the elongation of the cells located on the upper side of the embryo, the side adjacent to the suspensor. These embryonal tubes may divide by vertical walls before elongation, thus contributing, when they elongate, suspensor-sections made up of 2 or more collateral tubes. The early suspensor additions are formed in distinct sections, but as the embryo becomes multicellular and the divisions of the cells become more irregular the suspensors gradually become massive multicellular structures. In *Picea* they are 4-celled from the first.

The rosette cells (r) usually divide about the time the first embryonal tubes (e_1) begin to elongate, and in *Pinus* and *Cedrus* each of these 4 cells may form a multicellular embryo (q). These rosette embryos may put out suspensors (see rosette region of Fig. 10) or they may abort in any stage. The 8 pine embryos develop by means of typical apical cell growth from the embryo-initial stage, and in the successful primary embryos this may continue until the embryo reaches a cell mass of about 500 cells. However, the apical cell vanishes before any of the body regions of the embryo are organized (Bucholz '18).

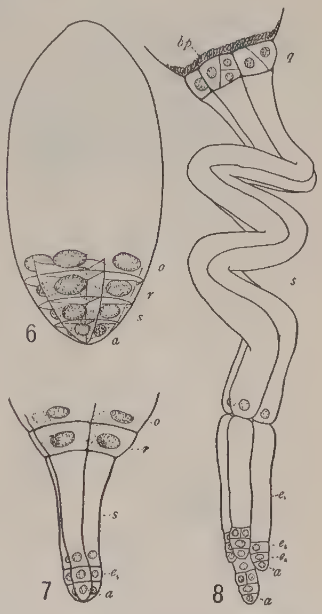

Fig. 5. Three further stages in development of early embryo in *Pinus*: *a*, apical cells; *s*, primary suspensor cells; *r*, rosette cells, which give rise to *q*, rosette embryos (latter usually develop later than in stage of embryo shown); e_1, e_2, etc., embryonal tube initial cells and embryonal tubes, which elongate and add to suspensor; diagrammatic reconstructions.

Cedrus repeats the early history of *Pinus* in nearly all essentials, (Buchholz '20) but with some recognizable differences. For instance, in *Cedrus* there is a longer period before the 4 vertical rows of cells constituting primary embryos become split apart into separate embryos but the cleavage is just as constant as in *Pinus*. *Tsuga* shows the apical cell growth in the primary embryo and likewise has the cleavage of the zygote, but the rosette cells abort in the unicellular stage.

Picea and *Larix* form only 1 embryo from each egg (Buchholz '20) and the rosette cells are only vestigial embryo initials which collapse very early. *Abies* is similar, but its embryo has been found to undergo cleavage in a small per cent of the cases investigated (Buchholz '26). *Pseudotsuga* is more unique for it has no rosette cells, or if it has, it is the tier of rosette cells which elongate regularly as pro-suspensors (Buchholz '20).

Taxodineae and Cupressineae

With the exception of *Sciadopitys*, these 2 groups of conifers are characterized by the existence of an archegonial complex; a group of archegonia very

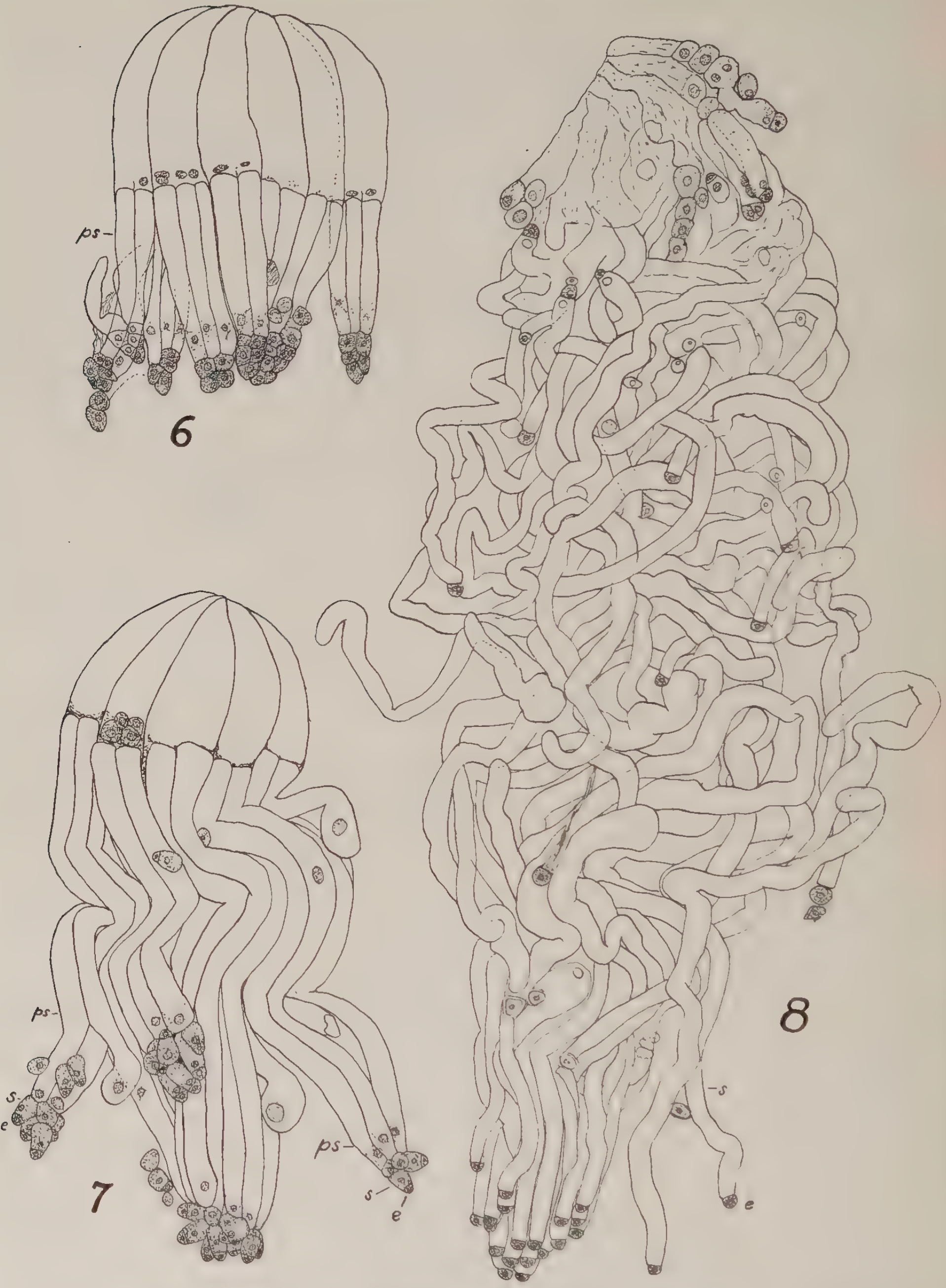

Fig. 6. *Biota orientalis* (*Thuja orientalis*). Archegonial complex showing pro-suspensor (*ps*) from many eggs beginning to elongate, each thrusting a group of embryo initial cells into prothallium below. ×175.

Fig. 7. Same as Figure 6 at a later stage. Pro-suspensors (*ps*) are longer, and most of the embryo-initial cells have divided to form primary suspensor cells (*s*) which elongate immediately and primary embryonal cells (*e*) which remain inactive for a time. Some embryo initials are aborting in this stage. ×175.

Fig. 8. Same as Figure 6–7 at a later stage of development. Primary suspensors (*s*) have elongated enormously, some aborting however. The embryo systems from different archegonia have become confused with each other, rendering the interpretation difficult without recourse to stages of Figures 6–7. See diagram of a single embryo system in Figure 9. ×175.

closely associated with each other and surrounded by a common archegonial jacket. It was shown by Strasburger ('69) that under these conditions a single pollen tube which contains at least 2 sperm cells may fertilize more than 1 egg.

The embryogeny of *Taxodium* was described by Coker ('03) and I have made no first hand observations on the embryogeny of this species. His work clearly demonstrates the occurrence of cleavage polyembryony in this form, with some indications that a brief apical cell stage is found in the early embryo.

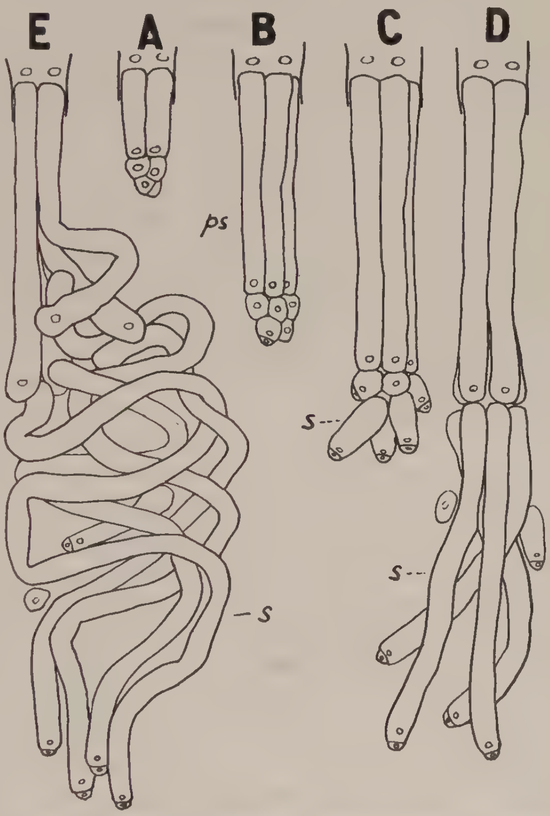

Fig. 9. Diagrams of steps in embryogeny of a single zygote of *Biota*. Explanation in text.

Strasburger ('72) has described the embryo of *Juniperus communis* and Noren ('07), the proembryo. The former's figures show the apical cell stage in this species, and from his figures, I would expect the features of the embryogeny which involve cleavage to be somewhat similar to that of *Biota* which I have investigated and will describe. *Biota* does not show the apical cell stage as clearly as *Juniperus communis*, and for this reason we may look upon the latter as being more primitive in this respect than *Biota*.

Biota has a large number of archegonia combined in an archegonial complex. This number usually varies between 9 and 15, but has been found to exceed 22. In the material which I observed most of the archegonia were producing embryos, and the resulting embryo complex is illustrated in Figure 8

in which are shown about 40 very young embryos participating in the competition.

The steps in the development of this type of embryo complex may be followed most satisfactorily by means of a diagram in which only one of the zygotes is considered. Figure 9 shows such a diagram. Here A represents the suspensors of a single one of the zygotes beginning to elongate. The suspensors shown here no doubt represent embryo initials which have changed in function to that of elongating cells; they are pro-suspensors, at least they are suspensors coming from cells organized in the proembryo. These pro-suspensors push the group of embryo initials into the enlarging cavity of the gametophyte. As the pro-suspensors elongate the terminal embryo initials divide. In Figure 9, B and C represent this condition. Almost immediately the larger proximal cells so cut off form suspensors which correspond to the primary suspensor cells in *Pinus*. For a brief period these make up a view of the embryo resembling in its essentials that which Arnoldi ('00[a], Buchholz '20[a]) figured for a stage of *Sciadopitys*.

In Figure 6 the several zygotes of an archegonial complex may be observed, each in the stage shown diagrammatically in Figure 9 A, and Figure 7 shows them in the stage represented in the diagram by B and C.

The stages represented by C and D, Figure 9, are passed through rapidly, so that we soon find the embryo systems in the stage represented in E, and this condition is found in the embryo complex of Figure 8. Here the confused mass of suspensors, pro-suspensors, and embryonic cells makes it difficult to trace out these relationships, but dissection sometimes results in the separation of the upper portion which includes the pro-suspensors from the suspensor mass of the lower portion.

In the region of the egg above the pro-suspensors, other cell divisions occur in later stages which give rise to an embryonic cell mass of abnormal appearance. Perhaps these are structures comparable to the rosette embryos of the Abietineae. This region is difficult to observe and these secondary embryos have not been studied in detail. It is possible that this appearance is partly due to new embryos arising from the belated fertilization of other eggs, but some of the figures of Noren on *Juniperus* (Noren '07) which show a proliferation of cells in this region suggest that these embryonic cells may be derived from the free nuclei which lie above the pro-suspensor cells.

Occasionally some of the nuclei within the pro-suspensor cells themselves undergo a further belated division. I take this to indicate that the pro-suspensors are really potential embryos; that they represent embryo initials in which the elongation activity was substituted for their normal embryo development, and it is for this reason that I am calling them pro-suspensors.

Libocedrus is very similar to *Biota*. Therefore the differences observed in this form are not fundamentally important. I have observed that some of the secondary embryos which appear in the rosette region of *Libocedrus* proliferate with distinct apical cell growth. Figure 14 shows one of these.

During the stage in which the primary suspensor cells of *Biota* undergo great elongation, the embryonic cells at their tips are enclosed by a double cell wall,

as shown in Figure 11a, a photomicrograph. The outer wall is very thin and attaches these cells to the suspensors, while another thick inner wall is organized around the protoplast within the outer wall. During this period the embryonal cell is relatively inactive, and when it resumes its growth the first cell division is nearly always vertical or slightly inclined. This terminal embryonic cell also divides, in the first steps, by apical cell growth, the primary suspensor representing the first segment.

Other divisions appear with inclined walls suggesting several segments, and the thick inner wall becomes thinner, while the embryo becomes an enlarged multicellular mass. There may be, therefore, a brief apical cell stage up to this point, but it is certainly confined to a very short period of development. Strasburger ('72) shows a well marked apical cell stage in *Juniperus* which is probably more primitive in this respect, but it seems to agree with *Biota* in the proembryo and probably in the method of cleavage polyembryony.

After the embryos have become multicellular some of them may break up into fragments. A many-celled embryo may sometimes become distinctly lobed or divided, as shown in Figure 12, the lobes or divisions sometimes separating from each other in blocks as bud fragments. This results, therefore, in a secondary cleavage stage in the embryo, but there seems to be nothing very regular about this second cleavage. Embryos may be produced without forming bud-fragments or they may be produced from one of the bud fragments of a former embryo. This secondary cleavage takes place at a stage when embryonic selection has been interrupted, when the primary suspensors are found collapsing, and before the secondary suspensors have appeared.

Thus far these embryonic cell masses have proliferated without producing additional embryonal tubes to form suspensors. A new phase of embryo growth seems to be initiated when these embryos or the fragments produce embryonal tubes. When the latter appear they are not at first single cells, followed by others arranged in groups as in *Pinus*, or as Strasburger figured for *Juniperus*, but they appear irregularly in clusters over the proximal region. Thus a massive suspensor is produced, forming a sudden break in continuity with the very long one-celled primary suspensor which has persisted so long, but which is usually found to be collapsing before this time. As the secondary suspensor of embryonal tubes becomes longer and the embryo mass more multicellular, the appearance approximates that of a normal conifer embryo (Fig. 13), the forward portion having become rounded, and any irregularities of its surface due to incipient bud fragments having disappeared. I have not followed the embryo of *Biota* beyond this stage.

Libocedrus decurrens was studied in these stages but this species shows this phenomenon of late cleavage in the formation of bud fragments to a very slight degree only, and it also was not followed very far into the later stages of the embryo.

I regard this late cleavage in forming bud fragments as a new development, not a palingenetic feature, as in the case of the first (proembryonic) cleavage of these species, and it has no counterpart in the other conifers which I have

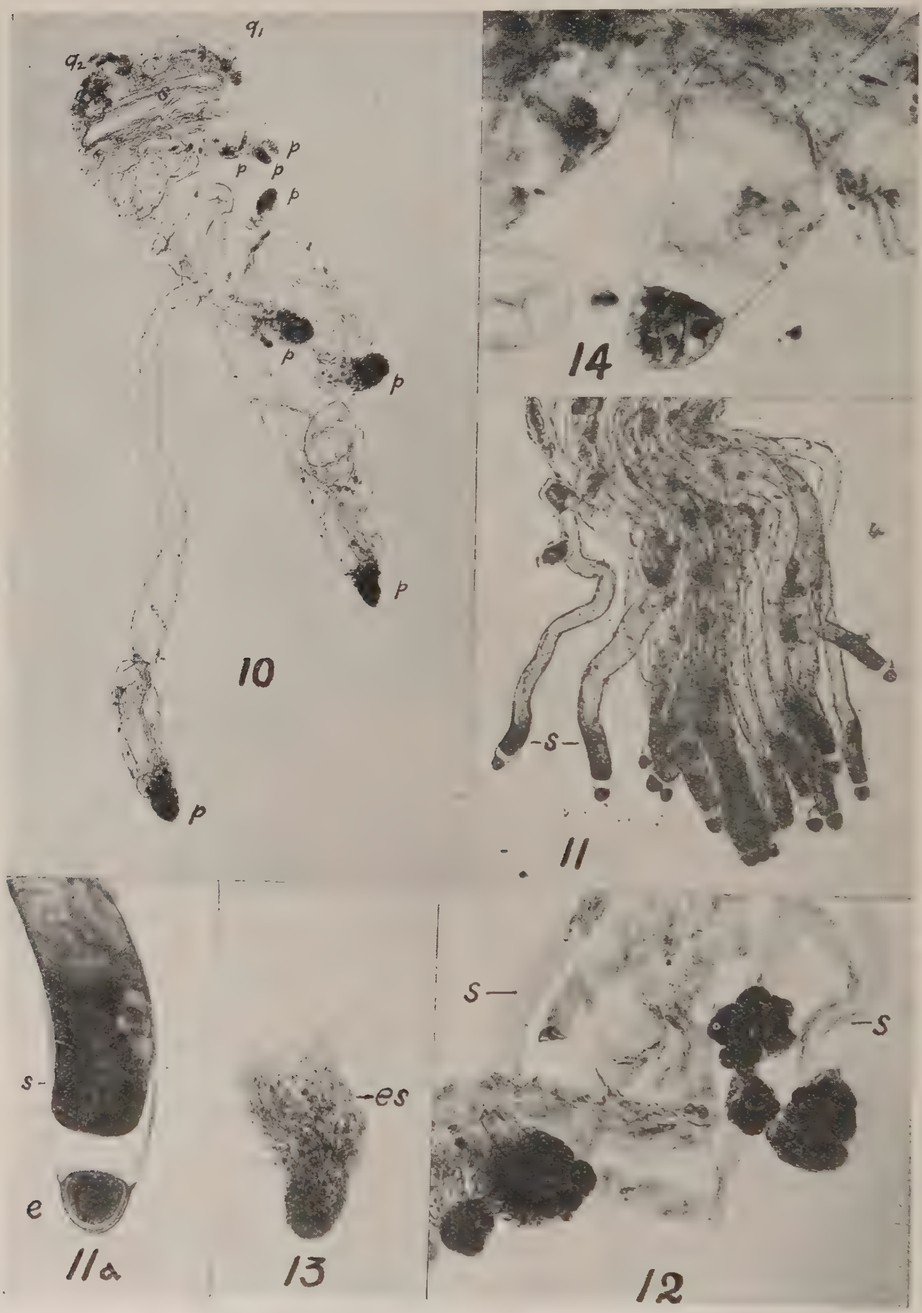

Fig. 10. *Pinus Laricio.* Photomicrograph of 2 embryo systems showing 8 primary embryos (p) below the collapsed folds of the primary suspensor (s) and several rosette embryos with elongated suspensors at q, others without elongated suspensors at q_2. $\times 40$.

Fig. 11. *Biota orientalis.* Photomicrograph of the lower end of an embryo complex similar to the one in figure 8. Twenty or more embryos are found, each separate on its own suspensor. ×90. Figure 11a is one of these more highly magnified showing double wall surrounding embryonal cell in this stage. ×440.

Fig. 12. Much later stage of *Biota* after embryonal cell has given rise to multicellular masses which may divide in a second cleavage stage as buds or bud-fragments. The 2 largest of these are combining the lobes into a single embryo and beginning to form secondary suspensors. ×120.

Fig. 13. Later stage of *Biota* (lower magnification) showing massive secondary suspensor (*es*) of many embryonal tubes with disappearance of nearly all buds or lobes. ×40.

Fig. 14. Embryo from rosette region of *Libocedrus decurrens* showing distinct apical cell growth. ×280.

studied. However, in *Gnetum* which was recently studied by Miss Haining ('20) we have a very good example of a parallel to this form of development, where there seems to be a similar variability.

At this point it will be appropriate to call attention to the similarity of the Gnetales to the conifers in their embryogeny. *Ephedra* may be derived from a condition between *Pinus* and the Cupressineae, from a stage in evolution in which separate archegonia still existed but in which the opposite or whorled leaves so characteristic of all Cupressineae had become fixed. The character of the proembryo fits in very beautifully as a derivative of some form having an early embryogeny resembling that of *Biota*. The proembryonic cleavage polyembryony identifies *Ephedra* at least, with the Abietineae or Cupressineae, and the later budding phenomenon associates *Gnetum* with the Cupressineae, though this may be due to a parallelism. Doubtless a careful morphological comparison will lead to the discovery of other structures showing agreements between the Gnetales and these conifers, and since cleavage polyembryony is also found in podocarps, it is not impossible to associate Gnetales with the podocarp group. The fact of greatest importance with reference to the Gnetales is that through their embryogeny they are more directly related to conifers than to other gymnosperms.

The embryogeny of *Thuja* is very different from *Biota*, though taxonomists have seen fit to merge these 2 genera on account of their superficial similarities. *Thuja* has only about 6 archegonia in the archegonial group. The completed proembryo is practically the same as that of *Biota* but the cells all contribute to form a single embryo. Figure 15 illustrates the embryogeny of *Thuja*. There is usually no cleavage polyembryony, a condition confirmed by my own studies.

Thuja has a distinct apical cell method of growth. Probably it was derived from a form like *Juniperus communis* which has an apical cell in this early period. When the separate embryo initials were combined, and the lowest cell of the proembryo in an advantageous position developed by apical cell growth, this device or method of growth was instrumental in coordinating all embryo initials into the formation of a single embryo. That the apical cell is found in *Thuja* was shown by Strasburger ('72) who traced it into a well-developed multicellular embryo (Fig. 15) where it disappeared as it does in *Pinus* before the tissue regions of the embryo are organized. I have no certain evidence that rosette cells are ever found, so there is at present very little vestigial evidence which indicates the origin of *Thuja* from a condition of cleavage polyembryony.

We may make this inference largely on the basis of the organization of the proembryo, and on the basis of Land's ('02) study showing occasional cleavage polyembryony in this form.

Of the other Cupressineae little is known save what Saxton ('10, '10[a], '13, '13[a]) has described for *Actinostrobus*, *Callitris*, *Widderingtonia* and *Tetraclinis*. His account of *Callitris* and *Actinostrobus* shows clearly the existence of cleavage polyembryony in these 2 forms but he did not describe it definitely for *Widderingtonia*, which may resemble more nearly *Thuja*. In *Callitris* he figured a thick wall surrounding the early embryonic cell and mentioned a period of inactivity for this cell, so that we may perhaps interpret this as a condition similar to that found in *Biota* (Fig. 11) and *Libocedrus* during the period of primary suspensor elongation.

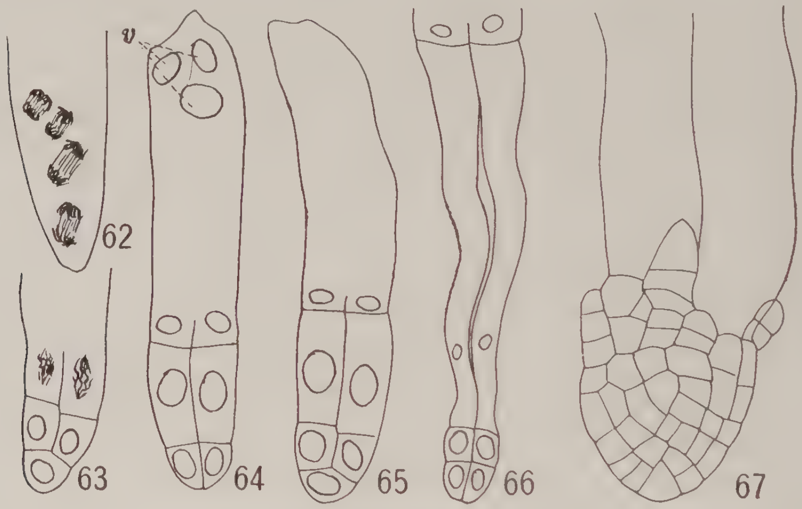

Fig. 15. Stages in embryogeny of *Thuja occidentalis*. Stages 62–66, proembryo and early embryo, after Land, ×300. Stage 87. Later embryo showing apical cell. ×225, after Strasburger ('72).

Among other Taxodineae which have been studied Lawson has described *Cryptomeria* which is probably more or less similar to *Taxodium* (Coker '03). A few stages of *Cunninghamia* and several other forms have been described, and the writer ('20[a]) has attempted to summarize elsewhere the work of several investigators on *Sequoia*.

Araucarineae

The araucarian embryogeny has been very fully described by Strasburger ('79), Eames ('13) and Burlingame ('15). Several stages are represented in Figure 16. Dr. Eames kindly placed some of his material of *Agathis* at my disposal and from this I have obtained only similar views of this type of embryogeny which these investigators have described quite fully. Here is an instance where the method of dissection does not offer great advantage over the methods of sectioning.

However, there is great difficulty in accepting any interpretation which regards the embryo of araucarians as a single unit. If this type of embryo represents only 1 embryo rather than a group of proembryonic initial cells combined, we must admit that this embryo has an endogenous origin, for which there is no parallel in the embryogenies of vascular plants. The embryogenies of certain podocarps and taxads afford the only comparisons, and I ('25) shall presently point out that there is good evidence that the latter are composite in their origin. The embryonic cap of araucarians therefore constitutes some evidence that these forms have passed through a stage in their history when they possessed cleavage polyembryony, the organization of the cap representing the method by which this polyembryony was brought back to the simple condition.

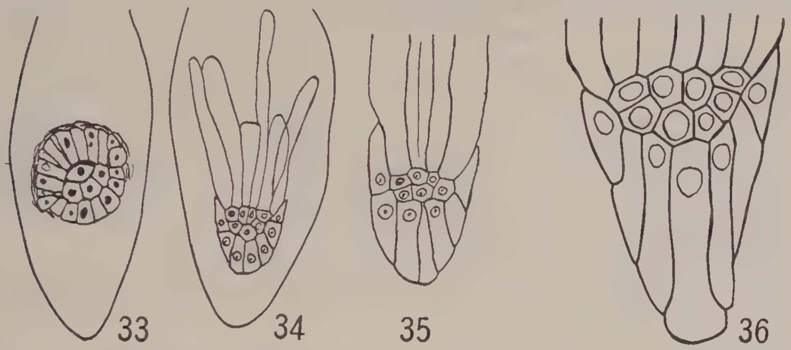

Fig. 16. Stages in embryogeny of *Agathis australis*. Stage 33. Proembryo after wall formation, ×200; 34, after cap is formed and pro-suspensor begins to elongate, but before archegonium is filled, ×260; 35, section through tip of older embryo showing the small cells below the pro-suspensor from which the embryo is derived, ×460. After photomicrographs by Eames ('13). Stage 36, embryo of *Araucaria brasiliensis*, drawn to half the scale of 35, ×230. After photomicrograph by Burlingame ('15).

Even with this explanation, the embryogeny of araucarians represents a high degree of specialization of a compound embryo and stands out in distinct contrast to the embryogeny of other conifers. There is evidence for the conclusion that there has been a long separation of this group from the other conifer lines.

Podocarpineae

The lowest proembryonic cells of podocarps are bi-nucleate. This fact has been observed and figured for *P. coriacea* by Coker ('02), for *P. nivale*, and *P. totara* by Sinnott ('13), who also mentioned this condition for the terminal cap cell of *P. spicatus*. In going over this literature and the slides prepared by dissection from some of Sinnott's material, I have found that the bi-nucleate condition of the proembryonic or early embryonic cells exists in all species of podocarps which have been examined: *Podocarpus nivale*, *P. totara*, *P. spicatus* (Fig. 19), *P. dacrydioides*, *Dacrydium cupressinum* (Fig. 17), and *Phyllocladus alpina* (Fig. 20). Thus without a known exception the early embryonic cells, which doubtless represent embryo initials, or the first division of these, are bi-nucleate in the podocarps. This bi-nucleate condition was not found in *Microcachrys* and

Pharosphaera recently described by Lawson ('23, 23a) who also concluded on other morphological grounds that these two genera belong elsewhere than among the podocarps. I believe this binucleate condition of the early embryonic cells in podocarps constitutes a distinctive diagnostic feature for this group.

If these bi-nucleate cells are embryo initials or their derivatives, as it seems likely, they are probably held in a condition of delayed differentiation by this means. The suspensor cells, and rosette cells when these are found, are not usually bi-nucleate, but of these units at least the suspensors are diverted early to the formation of pro-suspensors taking on a different physiological function, and the rosette cells may abort before reaching the bi-nucleate stage. It is certain at least that the further differentiation of the embryo is actually suspended during the period of the bi-nucleate condition, for they usually remain inactive during the period of a tremendous elongation of the suspensor (Figs. 17, 19).

Dacrydium has an interesting embryogeny, probably distinctive as a type. Of the proembryo I have no information, but at the time when the pro-suspensor cells elongate (Fig. 17 A) a group of 5 or more binucleate embryo initial cells are found at the tip. These bi-nucleate embryo initials are inactive for a considerable period during the elongation of the pro-suspensor (Figs. 17 A-C). During this period of pro-suspensor elongation one or more of them may become aborted or crushed. When they resume their activity they form multicellular masses (Figs. 17 D-F), some of the embryos aborting in this stage. In the further development the terminal embryo nearly always takes the lead. By the elongation of a secondary suspensor of embryonal tubes from its upper or proximal end, the terminal embryo pushes the remaining group of embryos, together with the pro-suspensor, back towards the micropyle (Fig. 17 G).

Thus the terminal embryo develops into the seed germ much as in other conifers and the other embryos belonging to the same system become crushed and aborted.

Sinnott ('13) observed that invariably the embryo buds or divides, in both *Dacrydium cupressinum* and *D. Bidwillii*. This series of dissections of the former species shows that this cleavage polyembryony may be traced back to binucleated embryo initials organized either in the proembryo, or during the earliest stages of pro-suspensor elongation.

A slightly different type of embryogeny is represented by *Podocarpus coriacea* (Coker '03). At first, there is only a single bi-nucleate terminal cell which divides vertically to form several similar units during the period of pro-suspensor elongation, each of these giving rise to a distinct embryo on the end of a pro-suspensor. The suspensor cells begin their elongation as unicellular structures, rather than by forming the more sudden massive secondary suspensor of many collateral embryonal tubes found in *Dacrydium* (Fig. 17 G). Figure 18 represents very diagramatically what one would find in such an embryo if it could be dissected and spread out. This figure is schematic and by no means a camera drawing, as are my other figures. My dissections of the material very kindly furnished by Dr. Coker agree with this interpretation, as do also the figures

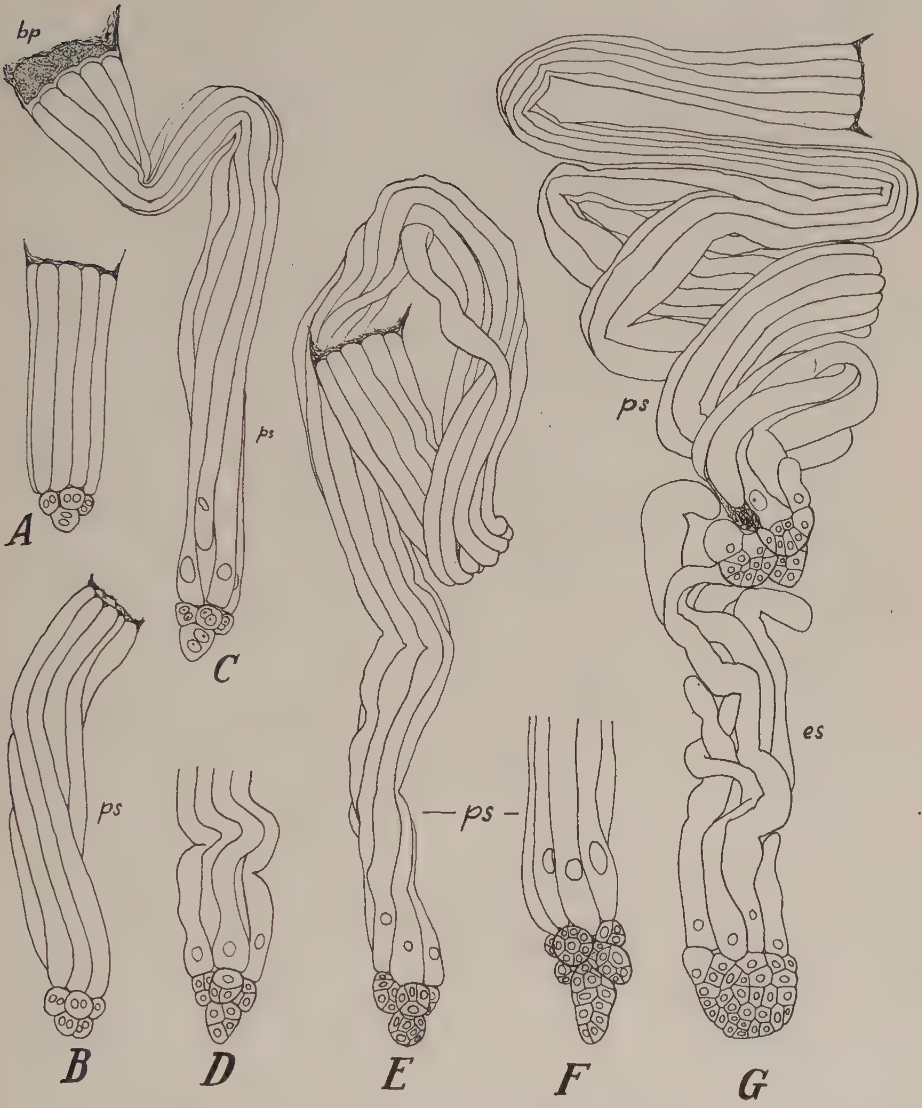

Fig. 17. *Dacrydium cupressinum.* A–C represent bi-nucleate stages of embryo during stages of pro-suspensor (*ps*) elongation. The plug (*bp*) in base of archegonium shown in C is dissected away in the other figures. D, F successive stages showing origin of separate embryos from bi-nucleate embryo initials. F has more embryos which may be due to a larger number of embryo initials. G. Later stage after massive suspensor (*es*) of successful terminal embryo has begun to push the group of smaller embryos (one of which has already aborted) and pro-suspensor upward toward micropyle. ×260.

which he ('03) described. *Podocarpus Hallii* (Sinnott's collection) appears to belong to this type.

I have also made a few dissections of early stages of the embryos of *Podocarpus totara* and *P. dacrydioides.* It is certain that the early embryos begin their development somewhat after the method of *P. coriacea* in which the terminal bi-nucleate cells are increased in number during the period when the pro-suspensor cells, elongate, but I have not found stages late enough to determine whether the several embryo initials combine to form a single embryo or whether they form several distinct embryos as in *P. coriacea* and in *Dacrydium.* I believe these will prove to be similar to the latter, judging from the tendencies of the cells to round off.

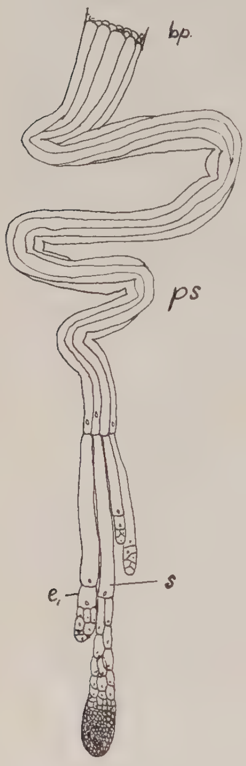

Fig. 18. Diagrammatic representation of a dissected embryo of the *Podocarpus coriacea* type showing relation of pro-suspensor (*ps*), primary suspensors (*s*) and secondary suspensors $e_1 e_2$ etc. Archegonium is at *bp* where "callose plug" (11) was removed by dissection. Region of suspensors (*s*) is a badly confused mass; the figure represents them as if they were spread out.

Podocarpus sicatus differs most widely in its embryogeny from the other Podocarps, and according to Sinnott ('13) who first described the podocarp embryo of this type, *P. ferrugineous* is very similar. Here we have a proembryo and early embryo so closely resembling that of *Cephalotaxus,* that one might easily be mislead into a very close association of these two types. These similarities represent only instances of parallelism in evolution.

In *Podocarpus spicatus* the proembryo becomes organized into a cap (a single bi-nucleate cell), a group of about a dozen to twenty bi-nucleate cells which form the single embryo, a tier of uni-nucleate pro-suspensor cells, and a few scattered rosette cells which usually collapse early, without giving rise to embryos. Figure 19 A shows this embryo after elongation of pro-suspensors has begun. The pro-suspensor cells elongate greatly before the bi-nucleate cells show further signs of differentiation, thus pushing the embryonic cells far into the prothallial tissue (Fig. 19 B). By the time these cells divide further and form walls, or long before this time, the cap cells are broken off (Fig. 19 C). Only one embryo is formed from each zygote. The two shown in A-C, Figure 19, represent in each case the products of two archegonia. As these bi-nucleate cells of the early embryo suggest, this embryo is doubtless composed of the fusion of several potential initials into a single embryo a condition in which the abortion of a few rosette cells and the existence of the cap cell, together with the parallelism to *Cephalotaxus* where this has happened (Buchholz '25) constitute the evidence of a derivation from a condition of cleavage polyem-

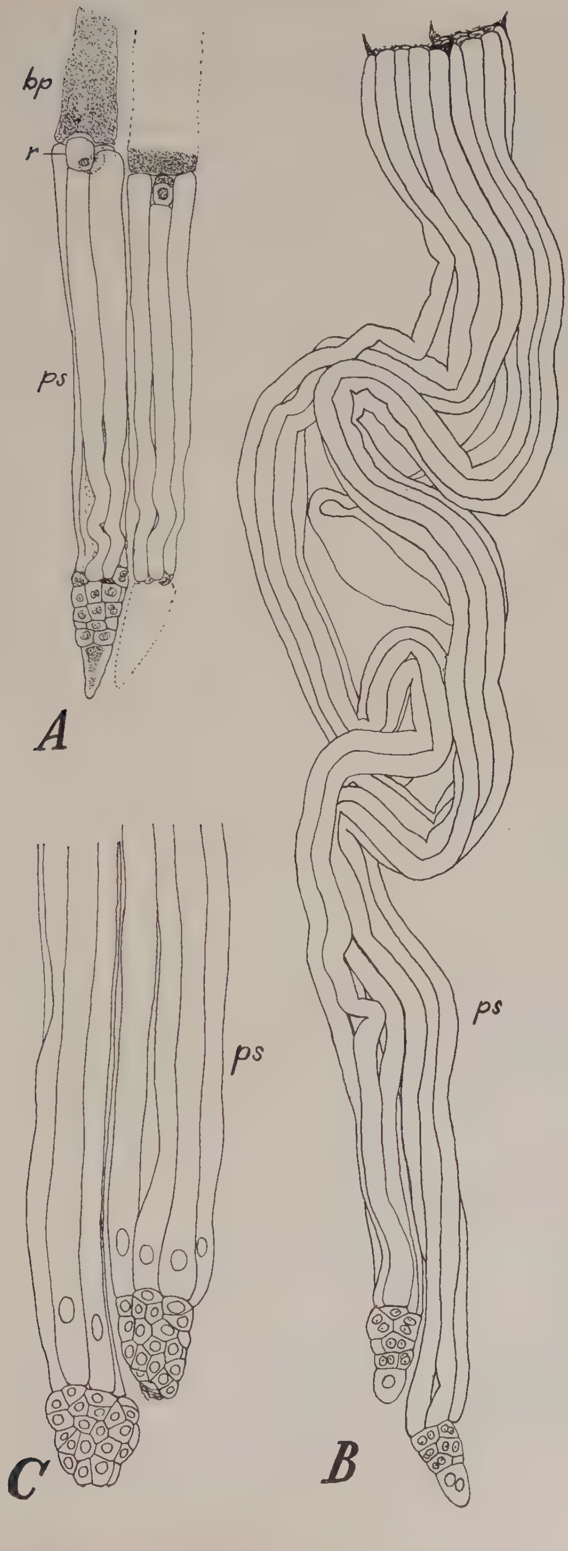

Fig. 19. *Podocarpus spicatus*. *A* represents stage when pro-suspensors (*ps*) have begun to elongate showing callose plug (*bp*) in base of archegonium, a few rosette cells at *r*, the group of bi-nucleate embryo initials below pro-suspensor, tipped by the aborting embryo initial which constitutes the cap. Missing parts of second embryo lost in dissection. *B* shows later stage with one of the cap cells showing bi-nucleate condition. *C*. Lower portion of older embryo in which cap cells have been discarded and bi-nucleate condition of embryonic cells has disappeared. ×150.

bryony. If we accept the other view that this zygote represents only a single unit, we must, on account of the deciduous cap cell, accept an endogenous origin for the region of the stem tip of the embryo, a condition which has no parallel outside of a few conifers.

Phyllocladus represents still another type of embryogeny. In the earliest stages which I have observed the pro-suspensors had begun to elongate (Fig. 20 A). The early embryonic cells are all bi-nucleate and the terminal cell is sometimes less deeply stained, suggesting that it might sometimes collapse after the fashion of the cap cell in *Podocarpus spicatus*. This terminal cell was actually found broken off in a number of cases, but this may have been due to the dissection. The suspensor does not become as long and twisted as in other podocarps, and upon further development after the bi-nucleate cells divide the increase in diameter of the embryo is proportionately as great as the increase in length of its suspensor. A few abortive rosette cells are found but no cleavage polyembryony.

The embryogeny of *Phyllocladus* is obviously nearest to that of the *Podocarpus spicatus* type and it also agrees with the latter in its very slender long-pointed archegonia. In this species, the archegonia are so long at the time of fertilization that they extend to the center of the gametophyte, so that the free nuclei of the proembryo migrate over a great distance in the cytoplasm of the egg to its tip, giving rise to the proembryo near this point at the center of the prothallium; a position reached by most other conifer embryos only after weeks of suspensor growth.

The apical cell in podocarps. In this group the apical cell method of growth of the early embryo does not seem to be as clearly represented as in *Pinus*. There is some evidence that the embryos of *Podocarpus coriacea* grow for a time by apical cell growth. This possibility is suggested by Coker's figures ('03), and in my examination of the dissected preparations of this species I have observed the cell arrangement characteristic of apical cell growth in some of the smaller secondary embryos which were being aborted. Though this apical cell stage seems to be only of brief duration, it occurs in the first stages and may be traced back to the bi-nucleate cells of the proembryo. It begins, therefore, from the first cleavages as far as any single embryo is concerned.

There is also a probability that an apical cell is found in *Dacrydium*, judging from the appearance of some of the early embryos on the tip of the pro-suspensor of Figure 17 D and E, and here it can be traced back to the bi-nucleate embryo intitial cells. However, its duration is also very brief for an embryo of the size of Figure 17 G does not indicate apical cell growth.

The embryo of *Phyllocladus* shows apical cell growth for what seems to be a slightly longer period. Segmentation is still suggested at the time shown by Figure 20 D when this embryo is composed of quite a number of cells (60 or more), but since this embryo represents from 6 to 8 or more bi-nucleate embryo initials combined, this stage actually represents only a few apical cell segments derived from the terminal embryo initial.

The embryos of *Podocarpus spicatus* show no signs of apical cell growth in

the early stages which I have observed. However, for a study of apical cell growth a very large series of stages is desirable both in sectioned material and in dissected preparations. My observations on podocarps are far from being as extensive as I should desire. This much seems fairly certain at least: that where the apical cell stage is found, it is of shorter duration in all podocarps than in *Pinus* and some other conifers whose embryos initiate their growth by this method.

Taxineae

The general facts of the embryogeny of 3 genera of taxads is known—*Cephalotaxus*, *Taxus*, and *Torreya*. In the first of these, *Cephalotaxus*, there is a strong resemblance to the embryogeny of certain podocarps. This resemblance is so pronounced that I ('25) have been mislead for some time by the apparent identity of the manner of embryo development in *Cephalotaxus* with that of *Podocarpus spicatus*. However, a more careful examination of material of both of these species has disclosed a fundamental difference between these 2 forms in that there is a uni-nucleate condition in the proembryonic and early embryonic cells in *Cephalotaxus* (likewise in *Taxus*, Jäger '99) and *Torreya* (Coulter and Land '05) and a bi-nucleate condition in the corresponding cells of *Podocarpus spicatus* which I have already discussed.

In the proembryo of *Cephalotaxus*, as in most other conifers, the proembryonic mass of nuclei and cells is crowded into the lower portion of the archegonium (Arnoldi '00, Coker '07, Lawson '07, '23). There are 16 free nuclei in the egg before any cell walls appear. In the next divisions, when these 16 nuclei give rise to 32, the walls are formed (Coker '07). This first multicellular stage of the proembryo may be regarded as a group of potential embryo initial cells (Buchholz '25). Most of these cells never function separately in forming embryos but give rise to other structures. Thus the lowest cell or group of 2, 3, or more cells goes to form the deciduous cap. The group of cells immediately above the cap are combined to form the single primary embryo, a tier of cells above this give rise to suspensor cells while the uppermost tier of cells forms a rosette. The group of cells which constitute the rosette is the only group of embryo initials which actually form several distinct embryos (Buchholz '25). The rosette cells have retained their separate embryo-forming capacity while most of the other potential embryo initial cells of the primary embryo have surrendered their individuality and combine to contribute pro-suspensors and the cell mass of a single primary embryo.

As the pro-suspensors elongate the cap cells are lost, the primary embryo rapidly becomes multicellular, and comes to lie deeply imbedded in the prothallial tissue. This mass of cells soon puts out a massive secondary suspensor of embryonal tubes from its upper or proximal end, which continues to elongate and become greater in diameter as the primary embryo becomes larger. Meanwhile, the rosette cells above the pro-suspensor group may proliferate, giving rise to rosette embryos with embryonal tubes, the only portion of the embryo of *Cephalotaxus* which remains primitive enough to exhibit cleavage polyembryony.

While the proembryo of *Cephalotaxus* is made up of more irregularly placed tiers, it shows a distant resemblance to some of the Abietineae in having a similar tier-grouping, with a rosette tier of embryo initials above, and in having the vestigial form of cleavage polyembryony exhibited by these rosette embryos (Buchholz '25). The deciduous cap cells represent something new, and we may interpret these cap cells as potential embryo initials diverted to form this organ, constituting a structure which doubtless facilitated the elimination of cleavage polyembryony among the group of cells which form the primary embryo.

The earliest account of the embryogeny of *Cephalotaxus* is that of Strasburger ('79). Arnoldi ('00), Coker ('07), Lawson ('07), and the writer ('25) have each added details to the account and this type represents one of the better known embryogenies of the Taxad group.

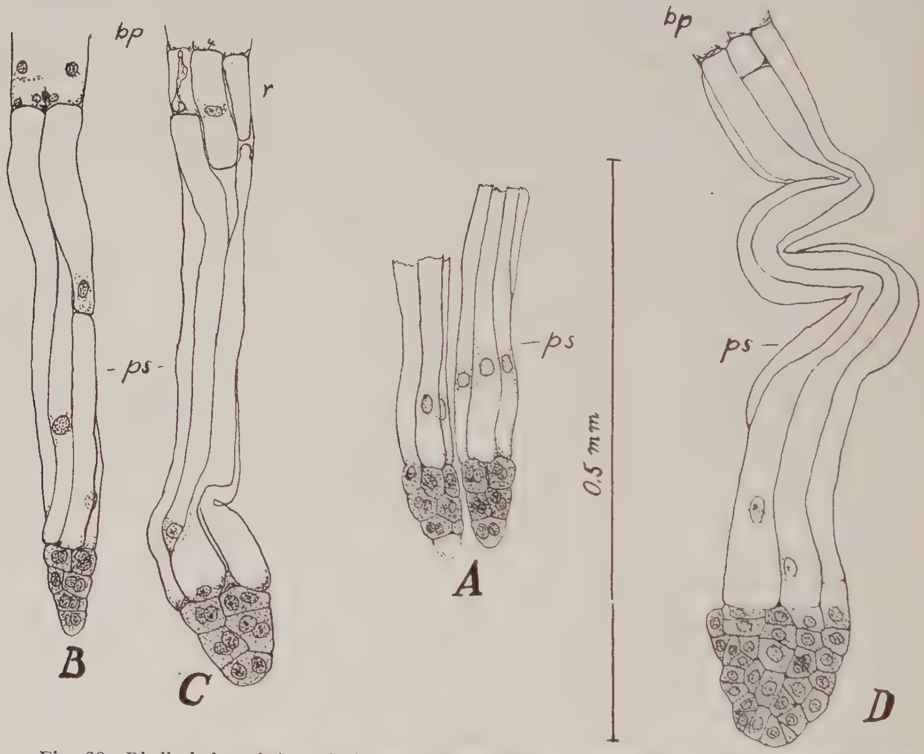

Fig. 20. *Phyllocladus alpina.* A–C, early stages of embryogeny showing bi-nucleate embryo initials below, pro-suspensor (*ps*) and base of archegonium (*bp*). *r* may represent rosette cells. D, older stage after suspensor has begun to coil, showing uni-nucleate stage and an apical cell which came from the lowest embryo initial. ×230.

The embryo of *Taxus* (Fig. 21) was probably not derived through the type represented by *Cephalotaxus*. It is characterized by simple polyembryony, but as Jäger ('99) states, in *Taxus baccata* it seems as though every proembryonic cell has the capacity of giving rise to an embryo. He observed and figured rosette cells (not always present and not shown in Fig. 20) and hinted at their

occasional division. He almost anticipated the essentials of my interpretation that the conifer embryo is composed of many embryo initials combined into a single embryo.

The embryo of *Taxus baccata* grows by means of an apical cell derived from the lowest embryo initial. This apical cell forms several segments as Strasburger ('72) has shown (stages numbered 39 and 40 of Fig. 21), and this is confirmed by Jäger ('99). It disappears sooner or later as the embryo becomes more multicellular, at a stage (Strasburger '72) still earlier, relatively, than in *Pinus*. Thus the apical cell stage, the occasional rosette cells or rosette embryos, and the occasional evidences of a tendency toward cleavages of the embryo suggest a primitive condition, while the embryo of *Cephalotaxus* is more specialized. *Cephalotaxus* shows no evidence of apical cell growth at any stage, not even in the rosette embryos, but the greater activity of the rosette cells suggests a more primitive condition than that found in the more vestigial rosette of *Taxus*. I ('20[a]) had previously placed *Taxus* in a more advanced position as if it were derived from a *Cephalotaxus* type; but after re-examining Jäger's account, I believe that we are justified in placing it on a par with or below *Cephalotaxus*. In the latter genus, the apical cell stage was probably eliminated along with the advent of the cap. Of course the long narrow archegonium of *Cephalotaxus* is also a more specialized condition, not shared in the history of *Taxus*.

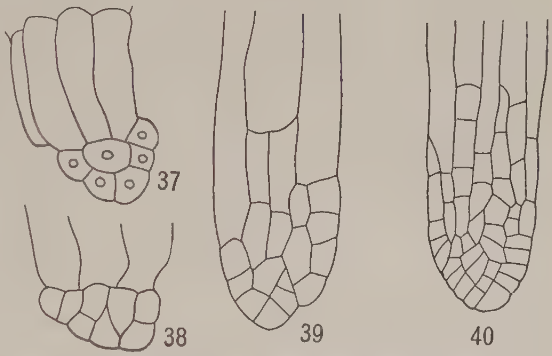

Fig. 21. Stages in embryogeny of *Taxus baccata*, ×125. Stage 37, Proembryo at beginning of pro-suspensor elongation, after Hofmeister; 38. Slightly later stage; 39 and 40. Later stages showing apical cell in early embryo. 38–40, after Strasburger ('72).

Toreya, described by Coulter and Land ('05), undoubtedly represents a more advanced type of embryogeny, probably derived through a form similar to Taxus. The proembryonic cells fill the entire egg and there is usually only a single archegonium, with a single embryo arising from each zygote. Its affinity within taxads is to *Taxus* rather than *Cephalotaxus*.

Discussion

In the above account I have attempted to give a review of conifer embryogeny with my interpretation, using a series of previously described and new examples of conifer embryo types. I have discussed the nature of embryonic selection and pointed out the manner in which cleavage polyembryony may have had its origin; that it represents a condition introduced very early in the history of conifer evolution, probably at the time of transition from ferns to seed plants, and that embryonic selection would tend to effect a return to simple polyembryony.

I have also attempted to show that in the development of a conifer embryo from that of a fern, the necessary modifications in the earliest embryo stages are not as radical as might be supposed; that these changes involve only such differences as might naturally be expected if a preliminary cleavage of the zygote into embryo initials and an enlarged egg are interpolated into the early embryogeny. It will be appropriate to discuss the morphological evidence which justifies this interpretation.

Apical Cell Stages

In the transition, ferns to conifers, the apical cell method of development was retained and I have regarded this as a palingenetic character, a fern feature still present in many living conifers. Other conditions being equal, one would therefore interpret a long period of apical cell growth in the embryo to represent a primitive condition, a short period an intermediate condition, and the entire absence of an apical cell in the embryo the most advanced condition.

Certain special conditions eliminated the apical cell earlier in some tribes or sub-families than in others. Thus the union of the four embryo initials of the same tier by the method found in *Abies*, *Larix*, and *Picea* doubtless eliminated the apical cell much earlier in this group than would probably have occurred otherwise, so that we may still regard this group on the whole as rather primitive, if other morphological facts justify this conclusion.

In taxads the elimination of the terminal embryo initial cells, as in the formation of the cap of the embryos in *Cephalotaxus*, is another special condition which would seem to render apical cell growth impossible, and if an apical cell stage were present when this change occurred, it was suddenly terminated. On the other hand, in *Taxus* the arrangement of the cells of the lowest tier in an uneven tetrahedral group probably facilitated or tended to prolong apical cell growth in the terminal embryo initial.

In most podocarps the apical cell stage is still found, though apparently for not so long a period of the ontogeny, and represents more nearly the intermediate condition of these embryonic apical cells. In *Podocarpus spicatus* the apical cell stage is not found, for the reasons which were given in the case of *Cephalotaxus*. Likewise in araucarians, no apical cell stage is found, for the cap of this embryo would doubtless prevent this method of growth.

In the groups Taxodineae and Cupressineae a marked apical cell stage of intermediate condition is found in the members whose embryo is most primitive, *Juniperus communis* for example; it is often represented by a few cell divisions only in the higher more specialized forms; but I find no certain instances where all traces of this method of growth have been lost. In *Thuja*, where reversion to simple polyembryony has set in, the apical cell is a conspicuous feature, probably for reason of being more conspicuous from the special grouping of the lower tier of cells as pointed out for *Taxus*. From all accounts and figures of the Gnetales they have no apical cell stage in the embryo and represent a condition so far removed from ferns that apical cells no longer exist, even though they have retained specialized forms of cleavage polyembryony.

In every case where the apical cell stage is found it originates directly from an embryo initial cell. For example, in *Pinus* it is at first a conical or hemispherical cell of 1 cutting face; after a few segments have been formed it changes to an apical cell of 3 cutting faces. In podocarps the apical cell stage, when present, follows the bi-nucleate stage, and, as stated before, each bi-nucleate cell probably represents an embryo initial whose nucleus has divided and remained in a condition of suspended or delayed differentiation. Therefore all instances of apical cell growth in embryos of conifers may be traced back to embryo initial cells.

CLEAVAGE POLYEMBRYONY

We may summarize at least 4 important lines of argument which may be pointed out in support of the theory that cleavage polyembryony had an early origin, or existed from the time of the transition to seed-plants.

(1) The orthogenetic effect of embryonic selection, which favors changes from cleavage polyembryony to simple polyembryony, renders it difficult for this condition to originate and establish itself. This was fully discussed in my opening paragraphs.

(2) In Abitineae, where the apical cell is known to be well represented, cleavage polyenbryony is associated with apical cell growth. The opposite interpretation, namely that the simple polyembryony of *Larix* and *Picea* is more primitive, implies that the apical cell did not exist continuously in Abietineae from the fern stage, but that there was, for a long period, an interruption in its existence during the stage when the embryogeny was in the *Picea* condition; it would leave unexplained the question of how such a method of growth might arise *de novo*. The more natural explanation is that the history of the apical cell is continuous. It was not eliminated to be re-introduced and later re-eliminated.

(3) The argument from rosette embryos and rosette cells, all of which abort sooner or later, consists of a complete reduction series in 3 or 4 parallel lines of evolution, several series which indicate clearly the direction of this polyembryonic evolution. Let us first consider the Abietineae in which I have previously stressed the importance of this evidence.

The rosette cells constitute a group of 4 embryo initials in Pinus, so that each zygote actually produces 8 embryos, 4 primary and 4 rosette embryos. In this genus the rosette embryos may develop more or less, even forming conspicuous suspensors (Fig. 10), though usually they abort earlier. Rosette embryos develop less on an average in *Cedrus* and usually abort in the unicellular condition in other Abitineae where rosette cells are found. Their occurrence in *Tsuga*, *Abies*, *Picea*, *Larix* and *Pseudolarix* can only be explained satisfactorily as a reduction series, a series of steps resulting from a gradual reduction and elimination of cleavage polyembryony.

The occurrence of rosette embryos in *Cephalotaxus* (Buchholz '25), occasional rosette cells in *Taxus* (Jäger '99) and their absence in *Torreya* represents another similar reduction series. The occasional rosette cells in certain podocarps represents a third reduction series, and some little understood events in the region above the pro-suspensors of *Juniperus* and *Biota* may perhaps furnish a

fourth similar reduction series for the rosette embryos. The opposite explanation that the rosette cells arose *de novo*, first as abortive cells which became permanent cells, and later as cells which produce only abortive embryos, would seem as absurd as the argument that the aborting ventral canal nucleus of an archegonium in Gymnosperms is more primitive than a ventral canal cell in ferns, or that the neck canal nuclei in the archegonia of *Pteris* are more primitive than the axial row of neck canal cells in *Lycopodium*. Thus we find that the most satisfactory alternative of these interpretations of rosette embryos and cells is that the condition of simple polyembryony has been derived from that of cleavage polyembryony, and that the latter occurred before the various living conifer types were differentiated as in the transition to siphanogamous seed plants.

(4) The embryos of practically all conifers show a compound nature in their organization, even those with simple polyembryony. This is shown by 5 conditions as follows: (a) by cleavage polyembryony found in various species; (b) by the rosette embryo reduction series just mentioned; (c) by the bi-nucleate organization of the early podocarp embryos, both those which do not split as well as those which split; (d) by the caps of araucarian and several other forms; and (e) by the variability in the number of embryo initials which may contribute to a compound single embryo.

Of these conditions (a), (b), and (c) have already been discussed. As for (d), I have also pointed out that if we interpret the embryos of araucarians, *Podocarpus spicatus* and *Cephalotaxus*, as single units we must regard these embryos as endogenous in origin, an unparalleled condition; at least the region of these embryos which later forms the stem tip must be considered unusual in being endogenous, a condition which has no counterpart in vascular plants. On the other hand, if we regard all of these embryos with caps as compound embryos derived from the union of many embryo initials, the cap structures come to represent abortive embryos and we need not assign to the embryonic cells an endogenous origin. That the latter alternative is the correct explanation is further indicated by the fact that in podocarps, where embryo units are recognized as bi-nucleate cells, these abortive cells are bi-nucleate; in taxads and araucarians where all of the embryo units or initials are uninucleate cells, these cap structures are composed of uninucleate cells.

Under (e) I have mentioned a variability in the number of embryo initials which may contribute to the compound single embryo. This may be observed in Figure 19 where the number of bi-nucleate cells below the pro-suspensors is not constant; it may also be observed in Figure 20; it has been mentioned (Jäger '99) as a characteristic of *Taxus* (Fig. 21), and of *Araucaria* (Burlingame '15), where the number of free nuclei at the time of wall formation is not fixed; it is no doubt found in many other conifers. The compound nature of these embryos would seem to justify this variability.

From these considerations we must regard an extended period of apical cell growth, cleavage polyembryony, and rosette embryos as primitive features; short apical cell stages and rosette cells which abort as more advanced; and the absence of apical cells, the condition of simple polyembryony, the absence of

rosette cells, and the presence of embryonic caps as the advanced features of conifer embryogeny.

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THE PERIODIC FRUITING OF DICTYOTA AND ITS RELATION TO THE ENVIRONMENT[1]

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At all places where studies have been made, all observed species of *Dictyota* produce the sexual cells in periodic crops at intervals more or less definitely related to the tides of each region. The relation to the tides varies, however, in different localities, and 3 different types of periodicity have been observed: 1 (recorded by Williams and Lewis) shown by *Dictyota dichotoma* at Bangor (Wales), Plymouth (England), and Naples (Italy); the second shown by the species of *Dictyota* resembling *D. dichotoma* occurring at several places on the coast of North Carolina; and the third shown by *Dictyota dentata* at Hope Bay, Jamaica. On the coasts of Europe the sexual fruits are produced in crops every 2 weeks, at times related to the spring tides of both the new and the full moon, the time relations of the crops to the spring tides differing, however, on each of the coasts of Wales, England, and Italy. Williams has shown that at Bangor, Wales, the plants are remarkably uniform in their reproduction, the great majority forming the rudiments of the sexual organs a few days before the least neap tide and liberating the sexual cells a few days after the greatest spring tide. At this place there are relatively great tides, the mean range of spring tides being nearly 7 m. and of neap tides about 3.5 m. At Naples, on the contrary, the tides are slight, the range of spring tides being only 30 cm. and of neap tides 15 cm. Here, as noted by Lewis, the plants are less uniform in fruiting. In fact, they are even less uniform than Lewis reported. In a third of the collections which I examined at Naples, I found male plants bearing fruits in all stages of development from undivided rudiments through mature and liberated gametes. The range of development was so great that it was only by tabulating my results in a calendar form that I was able to demonstrate satisfactorily the existence of periodicity at Naples. When this was done, however, it was evident that here, as at the other European places, the plants produce sexual fruits with a fairly regular rhythm at intervals of about 2 weeks.

On the coast of North Carolina, however, which has tides intermediate between those of Wales and Italy, at all places where *Dictyota* was found, the plants behave differently. Here they fruit not every 2 weeks at each set of tides, but every 4 weeks at the time of the full moon. Records of 28 crops made in 8 summers, over a period of 16 years, show that, in

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this region, *Dictyota* produces sexual cells only at the spring tides of the full moon, regardless of whether these are greater or less than the spring tides of the new moon. Here the plants disappear in the fall, no trace of them being found as late as the middle of May. Beginning their development soon after this, they grow rapidly and early in June have reached a length of 29 cm. and are ready to fruit. But, having attained maturity, they do not necessarily bear their crop at the next spring tide. Whether they fruit or not depends on whether the spring tide is that of the full moon or of the new moon. If the full moon occurs, they produce the sexual cells immediately, but if the new moon intervenes, they wait as many days as may be necessary until the time of the full moon arrives. The same condition holds for the plants growing throughout the season, these do not fruit immediately upon reaching maturity or at the first set of spring tides, but only at the times of the full moon. Moreover, unless retarded by unfavorable conditions, the plants produce their crops at the regular periods regardless of the actual tides to which they are exposed. Due to winds and other causes the tides frequently vary from the expected heights, in extreme cases low water of 1 series being higher than high water of another series, and yet the plants bear their fruits on the regular days that can be calculated for them. In no case was there evident any relation between the actual height of the tide and the actual days when fruiting occurred. The same fact was observed by Lewis and by myself for the plants of *Dictyota* at Naples. Slight variations from the regular period, usually of not more than 1 day, were found for different crops, for different plants in the same crop, and even for different parts of the same plant, but, altogether, the behavior is remarkably uniform. Of 22 times when the date of the liberation of gametes was accurately determined, 15 times showed this on the sixth day after the full moon, 3 times on the fifth, 3 times on the seventh, and 1 on the eighth day. While frequently single plants or parts of plants are slightly in advance or behind the main crop, about 80 per cent of the sexual cells are discharged in a single day and about 60 per cent to 70 per cent in a single half hour.

It is worthy of note that the time taken for the development of a crop (from the appearance of barely discernible rudiments to the liberation of the gametes) is, at the North Carolina points, only 6 to 8 days, compared with 10 to 13 days on the coast of Wales and 15 to 16 days at Naples. This fact may be related to the monthly fruiting at the former place compared with the fortnightly fruiting at the latter ones. It may be that, at the North Carolina points, the plants, during their long vegetative period, accumulate materials used in fruiting so that they are able to hasten the process when the fruiting periods arrive. It seems certain that this fruiting exhausts the plants here to an extent not observed at Naples and not reported elsewhere, the plants of this region, especially the females, frequently going more or less completely to pieces when they have discharged the sexual cells.

In Jamaica there is found a third type of periodicity. Here another species of *Dictyota* (*D. dentata*) shows its sexual fruits in a uniform state of development on all plants, most of these bearing two distinct crops of different ages, but it

so prolongs the development of the fruits that almost no change in the condition of the crop is noticeable during a period of more than 3 weeks. Of 36 fruiting sexual plants gathered throughout this period, all except 2 exhibited fruits mature or partly discharged and seemed no more developed on the last day than on the first. The evidence thus seems to indicate that, in Jamaica, there is no single day or group of days of general liberation of the sexual cells. A few plants of other species of *Dictyota* from other places in Jamaica have been obtained and indicate that this same condition obtains with them. Pending further studies in this region we may then form a tentative generalization, that, on the coast of Jamaica, various species of *Dictyota* bear their sexual fruits in regular crops, but so prolong the period of their development as to produce almost continuous fruiting, with a distinct overlapping of 2 successive crops, thus obscuring the regularity in the intervals of production. This fact may be related to the behavior of the tides in this region. Here the tides are practically negligible and highly irregular, the average range being 12–15 cms. with many days having no tides whatever. During spring tides the intervals between high and low water vary between 8 and 23 hours, while during the neap tide periods these intervals vary between 5 and 7 hours, or the tides are almost or entirely lacking. Perhaps this may account for the less regular, more prolonged fruiting conditions of *Dictyota* observed in this region.

A few sexual plants have been obtained from Bermuda. These show distinct evidence of periodicity, but the period is different from that found in North Carolina. Whether they have the period of any European localities or whether they have declared their independence and developed a period of their own can be determined only by further study. I am trying to obtain more material from Bermuda as well as from other regions, since only by extensive studies can we hope to obtain a clue to the meaning of the striking behavior shown by these plants.

Whatever the causes may be for the period fruiting shown by *Dictyota*, the plants retain their established fruiting periods for weeks or months after they are removed from tidal influences. At Bangor (Wales), at Naples (Italy), and at Beaufort (North Carolina), plants have been kept in the laboratory removed from tidal influences for periods of several weeks to several months, and in all cases produced the sexual fruits at the time characteristic of each region, even, in the plants of North Carolina, on new branches borne in the laboratory and therefore never exposed to the alternating influences of the tides. Similarly, plants which were placed in partly floating light and dark boxes and transferred so as to reverse the conditions of greater and less intense light normally experienced at spring and neap tides kept their usual periods of fruiting. In North Carolina the plants kept in the laboratory were always injured, the greater portions of them dying, the fruits being retarded or checked in their development. Some parts however, remained alive and frequently formed small side branches. All portions which remained living and capable of fruiting—the new branches which had never been subjected to tidal influences as well as the old parts—bore their fruits at the same period as did the plants growing under natural conditions.

Such plants in the laboratory were often 1 or 2 days behind the plants in the harbor in discharging the sexual cells, but this is probably to be ascribed to the effect of unfavorable conditions, since, in the fall, with the coming of unfavorable colder weather, the plants in the harbor may have their fruiting retarded for several days. The remarkable fact is that, under conditions that are so injurious as to kill the greater portions of the plants, the fragments that remain alive should still retain their fruiting period. Evidently this periodicity is very firmly impressed on the protoplasm of the plants.

Not only do the sexual plants mature the greater part of their fruits within a single day, but, at both Beaufort and at Naples they liberate the greater part of the reproductive cells within a single hour. The time of commencing the discharge of the eggs varies, in the laboratory at Beaufort, between 3:45 and 5:00 A.M. in different crops, even in the same season, and continues for about an hour. At Naples the 1 crop observed was discharged between 3:50 and 4:20 A.M. Sometimes liberation of the eggs began only after daylight was clearly evident, but most often it commenced before there was the slightest visible lightening of the Eastern sky. It would be interesting to determine the time of discharge of these plants in Jamaica, where the fruiting is less regular than in the other regions studied.

The relation between the inherent condition of the plants and the external factors regulating their periodic behavior is by no means clear, but the advantage of the periodic production of the sexual cells and their simultaneous discharge is evident, since the sperms are much more likely to reach the eggs and accomplish fertilization if both eggs and sperms are matured and liberated in large numbers within a single hour than if a few eggs and a few sperms are discharged at intervals throughout the month.

The non-sexual plants which alternate with the sexual ones in the life cycle show no periodicity, but produce their sporangia and discharge their spores constantly throughout the entire month. They also liberate their spores throughout the day and night, having no definite hour for discharging the reproductive cells. There is here a striking comparison between the advantage or non-advantage of a habit to the plants and the formation or non-formation of that habit by the plant.

Two questions now present themselves: (1) How can we explain the general periodic fruiting of the sexual plants of *Dictyota?* (2) How can we explain the monthly fruiting at North Carolina with its constant relation to the full moon?

While we cannot answer either of these questions, certain pertinent considerations present themselves. We find that whether in Europe or America, whether exposed to tides of 5–6 m. range (as in Wales), or to tides of 1 m. range (as in North Carolina), or to tides of 25 cm. range (as at Naples), wherever the tides are regular, *Dictyota* produces its sexual fruits in regular periodic crops on days that can be predicted within a slight margin of error. Where the tides are irregular (as in Jamaica) *Dictyota* prolongs the development of sexual fruits and overlaps the crops so that there is only a trace (but yet a distinct trace) of periodicity in its reproduction. At Bangor, at Plymouth, at Naples, and at Beaufort, the

tidal relations are different for each place, both in the range of the tide and in the hours at which low water of the great spring tides occurs, and in each of these places *Dictyota* has a different schedule for the initiation of the rudiments, for the time taken for the development of the crops, and for the day of liberation of the gametes.

From these facts it appears that there is a correlation between the fruiting periods of *Dictyota* and the tidal conditions to which it is exposed, and yet we find this alga continuing its periodic production of crops when entirely removed from tidal influence. At each of the places where experiments have been made (in Wales, Italy, and North Carolina) the plants in the laboratory removed from the influence of the tides keep the periodicity of their own locality, for example, the new branches formed under these conditions at Beaufort, North Carolina, fruit only at the times of the full moon.

What, then, is the explanation of this behavior of *Dictyota?* Williams believed it to be the greater amount of light received by the plants during the low water of spring tides; Lewis called attention to the fact that, at Naples, initiation of the, rudiments and liberation of the gametes begin when low water occurs at midday but notes that this explanation will not suffice for other regions. Neither of these suggestions will account for the fruiting behavior at North Carolina or Jamaica, and hence must be regarded as considering only 1 among many potent factors. We cannot assume that a phenomenon so deep-seated as this is called forth by 1 set of factors in one place and by another set in another place. Moreover, when, at Beaufort, unusual conditions cause variations in the tides, the plants fruit according to schedule and not according to the tides to which they are actually exposed. It seems that the fruiting behavior of *Dictyota* (and comparable behavior of other plants and animals) should be considered as truly a character of the plant (or animal) as is its form or structure. As the form and structure have been developed by the interaction of external conditions and the inherent capacity of the protoplasm, so with the periodic habit of fruiting. We can explain one to the same extent that we can explain the other, but no more. Neither is dependent on any single factor or group of factors in the environment, but is the result of the reaction of the plant to the sum of all the factors of the environment. The differences in fruiting at different localities would thus be accounted for by differences in the environment at these places, and also by the fact that, at each place, *Dictyota* is isolated from its relatives. As isolation frequently results in structural differences in both plants and animals, so it may result in differences in reproductive behavior when this behavior is a plastic character of the organism. The form and structure of *Dictyota* have been developed in the environment in which it grows, and would, we can surely believe, be different if the environment were sufficiently different. In fact plants growing under different conditions frequently assume unusual ("abnormal") forms and sometimes show structures bearing little resemblance to the usual *Dictyota* plants. In the same way we find that "unfavorable" conditions interfere with the usual regular fruiting and produce aberrancies in the crop. It appears significant that under unusual conditions, variations appear in both the form and the

fruiting habit, and these would seem to indicate that both form and habit are called forth by the reaction of the plant to the different conditions of its environment and that this is true in the one case in the same way that it is in the other.

While this phenomenon of periodic fruiting seems to have been developed by the inherent capacities of the plant, it seems that it must have been developed also in response to the factors of the environment. When we consider the close correlation between the fruiting periods and the external conditions, we are compelled to believe that these periods have been timed in response to the external conditions. It seems, then, that we have here a phenomenon produced in response to rhythmic external factors, yet maintaining itself for months when removed from these rhythmic factors, and, moreover, occurring in parts which have never been exposed to the rhythmic external conditions. It is greatly to be desired that culture methods may be devised in order that we may learn whether this fruiting habit is inherited by successive generations grown without the influence of these rhythmic external conditions.

The view proposed here is less satisfactory than those previously suggested in that it is less definite, but the facts seem to show that we are not warranted in making it more definite, since the behavior does not seem to be due to the action of any single, specific factor in the environment. This way of regarding the periodic fruiting does not preclude experimentation but does show it to be more complex and difficult than previously suggested, and it emphasizes the fact that experiments should take into account all the factors and not attempt the seemingly impossible task of accounting for the behavior in terms of the reaction to a single varying condition.

Other algae have been found to show more or less distinct periodicity in their sexual reproduction. Williams presented evidence suggesting the occurrence of this phenomenon in *Dictyopteris;* Wolfe showed that, at Beaufort, North Carolina, *Padina* produces its sexual fruits in fairly regular crops at intervals corresponding to the tides; Kuckuck observed that, at Helgoland, *Nemoderma* developed its gonads during each set of spring tides and liberated these during the following neap tides, and states that *Halicystis* produces its fruits in rhythmic succession, but at irregular intervals; recently Keefe has found that at Woods Hole *Sargassum* liberates its eggs in definite crops at irregular intervals.

It appears that the tendency to produce sexual fruits in periodic crops is fairly wide-spread, but further studies are evidently needed to show the relation between external conditions and the inherent capacity of the plants in the development of this habit of periodic fruiting.

The second problem, that of the relation to the full moon in the fruiting of *Dictyota* at North Corolina is still more difficult. This would be easier if we found that the plants fruited at the first spring tide after their development in May or June and then continued their fruiting at each alternate set of springs, but this is not the case. We have seen that, having reached maturity, they wait until the full moon occurs before producing the sexual cells.

Several animals are known to mature and liberate the sexual cells at more or less definite periods at times corresponding to the phases of the moon, usually near the time of full moon and often near the time of new moon. Such behavior has been described for 8 marine worms, 2 echinoderms, 1 mollusk, and 1 fish, while other animals mature their sexual cells simultaneously, but at irregular intervals.

From the observed facts five conclusions seem warranted: (1) The tendency to produce sexual cells at periodic intervals is inherent in many kinds of both plants and animals. (2) When such a tendency is present there is, at least in many cases, a further tendency to correlate the time of reproduction with regullarly recurring periodic changes in the external conditions. (3) The similarity in the behavior of different plants and animals indicates that these have correlated the periods of reproduction with similar external factors. (4) The changing external conditions with which the correlation of reproduction is most marked are the changing phases of the moon, or the changes in the tides effected by the moon. (5) In many cases the regular periodicity of reproduction has become fixed so that the periods are continued after the external conditions with which they are correlated are altered.

The periodicity does not seem to be due to immediate direct stimulation by external conditions, but does seem to have been developed in relation to such conditions. We can not conceive of such regular, rhythmic periodicity in such close correlation with rhythmic external conditions as having been produced independently of the conditions. We must, rather, believe that the plants and animals, having been exposed to regularly recurring changes throughout numberless generations, have acquired the correlation of their activities to these changes, and that, in some cases, this periodicity has become deeply impressed on the protoplasm.

The similarity in the reproductive behavior of some of the animals and plants is remarkable. In both groups we have some forms reproducing simultaneously at irregular periods, some forms reproducing at regular periods related to the full moon, and some forms at regular periods at other phases of the moon. In some cases the accounts of reproduction in animals could be used, with only slight changes of wording, to describe the facts as observed in *Dictyota*. Such striking similarity inevitably suggests a similar fundamental cause, but this suggestion can be established only after a more thorough study of all known cases of periodicity in reproduction and, perhaps, in other activities also.

Whether, in cases of lunar periodicity, we are concerned with correlation with the moon, or with tidal changes effected by the moon, we can not, at present, decide with complete assurance. It is, however, noteworthy that the periodicity in reproduction may continue irrespective of the tide, and that, in some cases, this occurs only at the times of the full moon. What, if any, may be the nature of the moon's stimulus is not clearly evident. The statements of authors regarding the nature of moonlight are contradictory, some asserting that it is largely polarized, others that it is polarized no more than sunlight. The question is complicated by the fact that sunlight at different times of the day and light of

both the sun and moon from different parts of the sky are polarized to different degrees. Acting on this suggestion, however, several workers have found that the growth of different kinds of bacteria and the hydrolysis of starch are greatly accelerated in the presence of polarized light. Whether these results have any bearing on the problem of reproduction at regular phases of the moon can be determined only by further experiment, but it is pertinent to recall that it is stated that a worm (the Atlantic Palolo) never swarmed when moonlight was prevented from falling on the rocks in which they lived.

The difference in the fruiting habits of the plants of North Carolina from those of *D. dichotoma* in Europe brings up the question of its systematic position. While morphologically resembling the European plants they have, as we have seen, a constant difference in the manner of fruiting, and under changed conditions, this fruiting habit is more constant than are the form and structure Whereas, under these circumstances, cell masses are frequently formed bearing little resemblance to *Dictyota*, the fruiting, if occurring at all, is only slightly delayed. Neither the form, nor the structure, nor the fruiting is independent of external conditions, but of these 3 the fruiting seems most firmly fixed. This being true, it seems that the fruiting habit is fully as much a character of the plant as are the form and structure, and should be equally considered in our classification. It seems, then, that we should regard the North Carolina plants as a different variety of *D. dichotoma*, resembling the species morphologically, but differing from it in its fruiting habit.

In conclusion, I may suggest that periodic production of sexual cells may be much more general than is realized and that the relation of this to phases of the moon may show us factors which are now scarcely suspected. We can not see what the future holds for us, but we can confidently believe that further studies of this habit will furnish us results of considerable importance.

PALAEOZOIC CONIFERS[1]

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One of the problems regarding the phylogeny of the Coniferales which has attracted particular attention during the last two or three decades is the question of the origin and relationships of the Araucarineae. A whole mass of literature has appeared, which has sought from different points of view and by various methods to throw light on this problem. A number of morphological, anatomical and embryological investigations of great value have been carried out, with the result that we possess an exhaustive knowledge of existing conifers of the genera in question, as well as of certain Mesozoic forms (compare Burlingame, Ghose, Gothan, Jeffrey, Kräusel, Pilger, Seward and Ford, Thomson, Torrey, etc.). Nevertheless, opinions on the phylogeny of the Araucarineae are as sharply divided as ever. A widely held theory is this, that the Araucarineae are beyond all doubt the oldest conifers, that they have been evolved from the Cordaitales, and that the Abietineae are to be derived from the Araucarineae as a relatively recent group. Other authorities, on the other hand, hold that the Abietineae are the oldest and that they have come direct from the Cordaitales, while the Araucarineae are believed to have evolved from the Abietinean stock in comparatively recent times. Further, there is a third view according to which both the Araucarineae and the Abietineae are very ancient groups and that both, independently, are derived from the Cordiatales in a broad sense. The lycopod theory, which associates the Araucarineae with the Palaeozoic Lycopodiales, we may leave out of consideration here (Seward and Ford; cf. also Scott).

Which of these views can now claim to come nearest the truth? There has been much contention on this point for many years. As Burlingame ('15) says in his review of the different opinions concerning "The origin and relationships of the Araucarians": "It seems rather unfortunate that so many of the facts known about gymnosperms may be used almost equally well to prove a variety of quite antagonistic views"; and again, to cite the work of Sahni ('20) ". . . . the controversy is certainly a beautiful example of the way in which practically the same data can be construed in diametrically opposite directions." It would take up too much space here to give an account, however brief, of all the arguments that the various camps are able to adduce in support of their respective theories. I propose only to dwell a little on the contributions to our knowledge of the oldest or Palaeozoic conifers which has so far been made. That these contributions are *a priori* of great interest from a phylogenetic point of view is self-evident.

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany, Ithaca, New York, Aug. 18, 1926.

The coniferous remains from the Permian and even the Upper Carboniferous formation described as *Walchia*, without doubt remind one, as far as the vegetative system is concerned, of *Araucaria*, or more exactly determined, of the section *Eutacta* of this genus. When Zeiller ('92) believed he had found a female cone of *Walchia filiciformis*, in which each scale bore a single seed, it was only natural that the genus *Walchia* came to be regarded as closely allied to the Araucarineae. In his admirable book "Studies in Fossil Botany" Scott ('23) says: "It has been maintained that Araucarieae are the oldest tribe of Coniferae; this statement, in the first instance, no doubt depended largely on confusion between Cordaiteae and Araucarieae; the former group is now excluded from the question, but there is some evidence, as we have seen, that in the Permian period conifers resembling Araucarieae both in vegetative and reproductive characters already existed." Concerning the age of the Abietineae, Scott believes that this group goes back at least as far as the Upper Triassic. But the direct palaeontological evidence is, in Scotts' view, favorable to the opinion that the Araucarineae have the longest history among conifers, probably overlapping that of the typically Palaeozoic family Cordaiteae. Other Palaeozoic genera too, such as *Ullmannia* and *Voltzia* (the Palaeozoic genus *Voltzia* is referred to below as *Pseudovoltzia*) have been believed in some degree to show Araucarian affinities.

As regards the Coniferous woods of the Palaeozoic, they cannot easily be distinguished from Cordaitean woods. As these latter further showed great similarity in structure to the Araucarian woods of the present day, the majority of palaeobotanists felt quite confident as to the great geological age of the Araucarineae. There was also the abundance of Araucarian cone-scales in the Mesozoic to be accounted for. For instance, in a work that appeared some years ago, Sahni ('20), writes: "While the palaeontological evidence places beyond doubt the existence of Araucarians in the Palaeozoic, the existence of Carboniferous and Permian Abietineae has been shown to be without proof."

Sahni refers this connection to a work on *Pityoxyla* by Jeffrey and Chrysler ('06), wherein these two authorities attached particular importance to two supposedly Palaeozoic woods, *Pinites Conwentzianus* and *Pityoxylon chasense*, and considered that they proved the existence of Abietineae as early as in the Carboniferous and in the Permian. These woods have since been examined by other palaeobotanists (Gothan, Seward, and Thomson and Allin), who came to the conclusion that the age of the former species and the determination of the latter probably are inaccurate.

For a long time the problem has remained at this stage. The one school of thought is convinced that the evolution has proceeded from Araucarineae to Abietineae, and the other school is equally certain that the contrary development has taken place. It seems desirable, therefore, if possible, to introduce into the discussion fresh palaeobotanical material of an indisputable Palaeozoic age.

For some six or seven years I have been engaged in preparing a monographic revision of the fossil conifers (with the exception of the woods), paying, of course,

special attention to the oldest known types from the Palaeozoic. Although this part of my investigation is at present far from completed, I think I am nevertheless already in a position to give a number of details of interest on the question of the relative age and the origin of the Araucarineae and the Abietineae.

To begin with, a brief description of certain more important genera may not be out of place. The material to be considered here can be suitably divided into the following groups: (1) Fertile or sterile leaf-bearing shoots. (2) Detached cone-scales. (3) Detached seeds. (4) Detached microsporangia. (5) Additional material of a more fragmentary character or not yet thoroughly investigated.

Even during later Palaeozoic times there seems to have existed at least about a dozen different coniferous genera; many of them we know very little about, as hitherto only sterile fragments and no traces of reproductive organs have been found.

Description of Material

I. Fertile or Sterile Leaf-Bearing Shoots

Lecrosia gen. nov.

Female cones ovoid, isolated, erect, terminal in postition, built up of an axis and numerous scales, the latter being spirally disposed, sessile, imbricate, linear-lanceolate, showing entire margin, elongated apex, and abruptly cuneate base. Scales each probably bearing 2 seeds, which seem to be furnished by a one-sided wing. (Whether the scales are simple or not cannot be accurately determined.)

Male cones not known.

Leaves needle-shaped, spirally disposed, linear-lanceolate, spreading but incurved in their outer portion, sessile, decurrent, and traversed by a single "vein."

Geographical distribution: Le Cros, near Saint Étienne, France.

Geological age: Upper Stephanian (cf. P. Bertrand '18).

Literature: Florin 1927, p. 2.

Walchia Sternb. emend.

Female cones cylindrical, isolated, erect, terminal in position on ultimate branches, showing spirally disposed, sessile, imbricated, spreading, linear-lanceolate bracts with bifurcate apex (*Gomphostrobus*) and in each of their axils one short-shoot with scales spirally arranged. The upper scales were probably, at least partly, fertile. Seeds platyspermic with narrow wing (*Cordaicarpus*). Sterile leaves on branches bearing female cones showing the same cuticular structure as sterile leaves on specimens bearing male cones, as well as on sterile specimens.

Male cones larger and cylindrical or smaller and ovoid, isolated, erect or pendulous, terminal in position on ultimate branches showing spirally disposed, imbricate, sessile, adpressed, hairy, lanceolate or ovate-lanceolate sporophylls

bearing microsporangia. Number and arrangement of the latter not known. Microspores with a broad ring-shaped air chamber as in *Cordaianthus*. Arrangement of stomata on ♂-sporophylls corresponding to the arrangement on sterile leaves.

Foliage shoots with a pinnate arrangement of the ultimate branches. Leaves on ultimate branches spirally disposed, imbricate, decurrent, usually tetragonal in transverse section, with a single "vein," hairy, most frequently linear, curved and somewhat spreading; but occasionally linear, almost upright and adpressed; or even short, ovate and adpressed to the shoot. Stomata arranged in two long, broad or rather narrow bands on the ventral side of the leaves and two shorter and narrower bands on the dorsal side. The dorsal side of the leaves sometimes entirely without stomata. Within each band the stomatal apparatus is irregularly distributed and very often possesses subsidiary cells partly in common with neighboring apparatus. Subsidiary cells most frequently showing papillae. Leaves on penultimate branches longer, broader, from a broad and decurrent base tapering to an acute apex, keeled, "uninerved," hairy, adpressed and almost straight or spreading and curved.

Several species.

Geographical distribution: Very widely spread in central, southern and western Europe. Occurring also in eastern Canada and Kansas, Texas, Oklahoma, Colorado, and New Mexico.

Geological range: Upper Stephanian (France)—Lower Permian.

Literature: Goeppert 1864–1865, p. 236; Bertrand 1918, p. 691; Bertrand 1919, p. 175; Bergeron 1884, p. 533.

Ernestia gen. nov.

Female cones cylindrical, isolated, erect, terminal in position on ultimate branches, with probably spirally disposed, overlapping, fanlike scales, which are deeply trilobate in the upper expanded part and contracted at the base, here bearing one single seed (?). Whether the scales are simple or not cannot be accurately determined.

Male cones ovoid, isolated, erect or more often pendulous, terminal in position on ultimate branches and showing spirally disposed, imbricate, sessile, adpressed, lanceolate sporophylls. Microspores with a broad ring-shaped air chamber (as in *Cordaianthus*).

Foliage shoots with a pinnate arrangement of the ultimate branches. Leaves on ultimate branches spirally disposed, decurrent, tetragonal in transverse section, hairy, finely dentate at the margins, linear or from a broad base tapering to an acuminate apex, curved, spreading generally at right angles from the shoot, but near the apex strongly curved towards the top of the shoot. Stomata on both sides of the leaf, arranged in longitudinally-running parallel rows of varying length, each row being generally only one stoma broad. Subsidiary cells papillate. Leaves on penultimate branches longer, broader, curved, spreading at right angles from the shoot.

Probably more than one species.

Geographical distribution: Widely spread in central, western and southern Europe. Probably occuring also in the central part of the United States.

Geological range: Lower Permian.

Literature: ZEILLER 1892, p. 99, pl. 15, f. 3.; Potonié 1893, pl. 27, f. 12.

Dicalamophyllum Sterzel

Reproductive organs not known. Leaves flattened, linear or linear-lanceolate, acute or obtuse, with somewhat involute margins, on the upper side convex, with occasionally a slight groove in the middle line, on the lower side showing three longitudinally running grooves, of which the middle one corresponds to the vein and the two lateral ones contain the stomata; transverse section showing a single bundle region invested by a rather distinct sheath; whether the vascular bundle was double or not cannot be determined.

One or possibly two species.

Geographical distribution: Altendorf, near Chemnitz, and possibly Saalhausen, near Oschatz, Saxony.

Geological age: Lower Permian.

Literature: STERZEL 1880, p. 13, pl. 2, f. 17–21; STERZEL 1886, p. 59, pl. 8 (28), f. 5 a-d.

Buriadia Seward et Sahni

Reproductive organs not known. Foliage-shoots sparsely and irregularly branched. Leaves spirally disposed, varying considerably in size and in the form of the lamina; leaves on the more slender branches linear acuminate, frequently bifurcate at the apex; leaves on stouter branches longer and obcuneate. Lamina decurrent, traversed by a single "vein" which forks some distance below the forked apex of the lamina.

One species.

Geographical distribution: Karharbari beds at Buriadi, India.

Geological age: Lower Gondwanas (Permo-Carboniferous).

Literature: SEWARD and SAHNI 1920, p. 12, pl. 2, f. 20–25a.

Morania Seward et Sahni[2]

Reproductive organs not known. Foliage-shoots thick, bearing large, spreading, spirally disposed, lanceolate, acuminate leaves which are slightly contracted at the base, acute at the apex, and characterized by numerous parallel striations. Actual veins not visible.

One species.

Geographical distribution: Moran River, India.

Geological range: Lower Gondwanas (Permo-Carboniferous).

Literature: ZEILLER 1902, p. 36; pl. 7, f. 6. SEWARD and SAHNI 1920, p. 14.

[2] According to information kindly supplied by Prof. B. Sahni, of the University of Lucknow, India, this generic name must be changed, as it has already been previously adopted to denote certain Middle Cambrian algae (Walcott).

Pseudovoltzia gen. nov.

Female cones cylindrical, isolated, erect, terminal in position on ultimate branches, with spirally disposed bracts and in each of their axils one fan-like ovaliferous scale, which is most frequently deeply fivelobated in the upper expanded half, contracted to a sort of petiole in the lower one and bear 2–3 ovate seeds on the lower part of the expanded portion. Whether the scales are simple or not cannot be accurately determined. Seeds with a narrow membranous margin. Sterile leaves on branches bearing female cones as well as female sporophylls showing the same cuticular structure as leaves on sterile branches.

Male cones not known.

Leaves on ultimate branches spirally disposed, spreading, decurrent, glabrous, dimorph; at the ends of the branches long, linear, flattened, obtuse; on the lower part of the branches shorter, slightly incurved, subtetragonal in transverse section, and obtuse at the apex. Stomata arranged on both sides of the leaf in numerous longitudinally-running parallel rows, each one being generally only one stoma broad. Subsidiary cells papillate.

Probably more than one species.

Geographical distribution: Frankenberg in Hesse, Germany; Trebnitz, near Gera in Saxony, etc.

Geological range: Upper Permian.

Literature: Geinitz 1880, p. 26, pl. 5, f. 1, 2, 5–8, 10–19.

Ullmannia Goepp.

Female cones cylindrical, isolated, erect, terminal in position on ultimate branches, with probably spirally disposed, somewhat overlapping, loosely arranged, sessile, entire, broad, orbicular or fan-shaped scales each bearing one large orbicular seed on the upper (adaxial) surface. Sterile leaves on branches bearing female cones showing the same cuticular structure as leaves of sterile twigs and sporophylls of male cones.

Male cones smaller, ovoid, isolated, with spirally disposed, imbricate, sessile, adpressed, triangular sporophylls bearing microsporangia. Number and arrangement of the latter not known. Microspores with two bladders as in recent Abietineous genera. Arrangement of stomata on ♂ sporophylls corresponding to the arrangement on sterile leaves.

Leaves on ultimate branches spirally disposed, crowded, glabrous, ovate or lanceolate, acute or obtuse, decurrent, often keeled, spreading or adpressed, somewhat flattened, radially built, traversed longitudinally by a single central bundle region, which shows a characteristic wing of transfusion tissue on each side. Stomata arranged on both sides of the leaf in numerous longitudinally-running parallel rows, each one being only one stoma broad. Subsidiary cells papillate.

Some few species.

Geographical distribution (during the Palaeozoic): Western, central, and eastern Europe.

Geological range (within the Palaeozoic): Upper Permian.

Literature: Solms-Laubach 1884; Gothan and Nagalhard 1922, p. 442.

II. DETACHED CONE-SCALES

Araucarites Presl.

Cone-scales broadly triangular in outline, cuneate and truncate at their base, rounded and with a small median depression at their apex, showing in the middle a shallow oblong depression, which has in all probability contained a single seed, and on each side of this depression a slightly depressed contour, possibly caused by a wing.

One species.

Geographical distribution (during the Palaeozoic): Charmoy, near Autun, France.

Geological range (within the Palaeozoic): Lower Permian.

Literature: Zeiller 1906, p. 215, pl. 50, f. 1.

III. DETACHED SEEDS

Pityospermum Nath.

Seeds detached, winged on one side and resembling recent Abietineous seeds.

Geographical distribution (during the Palaeozoic): Lodève in southern France; Trebnitz, near Gera, in Saxony; Fünfkirchen in Hungary.

Geological range (within the Palaeozoic): Lower Permian—Upper Permian.

Literature: Heer 1876; pp. 15, 18.

IV. ADDITIONAL MATERIAL

OF A MORE FRAGMENTARY CHARACTER OR NOT YET THOROUGHLY INVESTIGATED

Furthermore, the following sterile remains seem to represent separate genera not identical with any of those above-mentioned:

1. Some sterile specimens of a conifer found in the Coal-Measures of Brazil and described by White and Lundqvist have nothing to do with the European genus *Voltzia* Brgn., but belong to a new genus which will be described later on.

Geographical distribution: Rio Grande do Sul and Santa Catharina, Brazil.

Geological age: Permo-Carboniferous.

Literature: White 1908, p. 569, pl. 8, f. 11–13b; Lundqvist 1919, p. 21, pl. 2, f. 14–16.

2. *Walchia*-like sterile foliage-shoots whose taxonomic position is very uncertain.

Geographical distribution:

Geological age: Permo-Carboniferous.

Literature: Kurtz 1921, pl. 7, 13, and 27 (in part).

3. Foliage-shoots with spirally disposed, more or less scalelike leaves and small possibly male cones, terminal in position on ultimate branches and described under the name *Brachyphyllum* (?) *australe* Feistm.

Geographical distribution: New castle beds, N. S. Wales, Australia.

Geological age: Permo-Carboniferous.

Literature: Feistmantel 1878–1879, p. 97; pl. 7, f. 3–6, pl. 17.

4 *a*. Sterile foliage-shoots, *Walchia*-like in habit.

b. Trilobate female sporophyll (whether belonging to *a* uncertain).

Geographical distribution:

Geological age: Permo-Carboniferous.

Literature: Feistmantel 1880–1881, pl. 47 A, f. 19, 20, 22, 24; Seward and Sahni 1920, p. 17, pl. 2, f. 26.

5. Sterile shoots bearing leaves with dichotomously divided apex; isolated leaves with dichotomously divided apex as well (*Gomphostrobus* Marion). Probably all belonging to *Walchia*.

Geographical distribution: Spread in central, western and southern Europe and probably occurring also in the central part of the United States.

Geological age: Lower Permian.

Literature: Marion 1890; Potonié 1893, p. 197, pl. 27, 28, and 33 (in part).

6. *Piceites clekensis* Gein.—Geinitz 1880, p. 31, pl. 4, f. 19, 19a.

Geographical distribution: Iletzkaja Sachtschita, near Orenburg, Russia.

Geological age: Upper Permian.

7. *Ullmannia Solmsi* Goth. et Nagalh.—Gothan and Nagalhard 1922, p. 447.

Geographical distribution: Ilmenau in Thuringia and Mansfeld in Saxony.

Geological age: Upper Permian.

8. Foliage-shoots not yet described.

Geographical distribution: Trebnitz, near Gera in Saxony.

Geological age: Upper Permian.

9. The sterile specimens of conifers from Fünfkirchen, Hungary, described and figured by Heer (1876) under the name of *Voltzia hungarica* Heer and *Voltzia Böckhiana* Heer, seem to represent a new genus but of doubtful affinity (perhaps identical with No. 6).

Geographical distribution: Fünfkirchen, Hungary (cf. under No. 6)

Geological age: Upper Permian.

10. Among the coniferous remains from the "Grödner Sandstone" in the Eastern Alps I shall later on describe a new genus possibly of Araucarian affinity founded on sterile material with the cuticular structure preserved. This genus is possibly represented also in the Permian flora of Fünfkirchen, Hungary (cf. under No. 5).

Geographical distribution: Tyrol, Italy.

Geological age: Upper Permian.

Literature: Gümbel 1879, p. 73.

Conclusion

The question now presents itself, which of the Palaeozoic genera mentioned above may be considered to show Araucarian affinities and which may not.

The detached cone-scale described by Zeiller from the Permian of France, which have been attributed to the genus *Araucarites*, should probably, as far as

can be judged at present, be conceived as belonging to the Araucarineae. It is possible that the genus *Morania*, from the Lower Gondwanas should also be placed in the same family, although this is very uncertain. Some sterile twigs from the Grödner Sandstone of the Eastern Alps show a cuticular structure of the leaves which may possibly be conceived to be an Araucarian structure. The genus *Ullmannia* is characterized, it is true, by single-seeded cone-scales, but also by microspores with two bladders, as in recent Abietineae. Finally, the genus *Ernestia* with lobate, though probably single-seeded, cone-scales apparently do not belong to the Araucarineae.

Thus, quite contrary to the view usually held hitherto, Palaeozoic conifers showing undoubtedly Araucarian characters seem to be few in number and with only one exception somewhat doubtful. One has almost greater success by looking amongst the oldest known genera of conifers for characteristics generally conceived to be indicative of Abietinean affinities.

The genus *Lecrosia* from the Upper Carboniferous of France probably possesses two alate seeds on each cone-scale. It has further been possible to assign similar isolated alate seeds, which resemble those of the present-day Abietineae, to both the Lower and the Upper Permian. *Ullmannia* possesses as I have just mentioned, microspores with two bladders just as in the case of present-day Abietineae, although the cone-scales are single-seeded. The genus *Pseudovoltzia* is probably allied to *Ullmannia* but possesses 2–3-seeded, lobate ovuliferous-scales. The genus *Dicalamophyllum* in the Lower Permian, of which so far only sterile leaves are known, gives an undoubted impression of being more closely related to the Abietineae than to the Araucarineae.

The most remarkable of all Palaeozoic genera of conifers hitherto determined is undoubtedly *Walchia*, which predominates in the Lower Permian. All indications go to show that the female cones in this genus are inflorescences and show great similarity in structure to that of *Cordaites*. In fact, Schoute (1925) has just recently proved that female catkins of *Cordaites* are strobili arranged in inflorescences. The structure of the female cones in *Walchia* should, I think, be regarded as supporting the brachyblast theory, which holds that the female cone of the Abietineae is to be looked upon as an inflorescence, that is, an axis with bracts, in the axil of which are reduced secondary fertile shoots.

Which of the two families, Araucarineae or Abietineae, is the older cannot even yet be determined with any certainty. It seems to me not improbable that the Abietineae have evolved from the Cordaitales in a broad sense in view of their similarity as regards the structure of the female cones. To derive the Araucarineae from the Palaeozoic Lycopodiales offers serious difficulties, while a close relationship with the Cordaiteae is also hardly admissible. According to Scott ('23) Gordon has found a new species of the genus *Pitys* in the Lower Carboniferous rocks of Great Britain, which has thrown more light on the structural features of this genus and has rendered its correlation with the Cordaitales not very probable. On the other hand, the new species of *Pitys* shows many striking similarities with present-day species of *Araucaria*, section Eutacta.

Pityeae seem to form a very interesting family, rather independent of the class Cordaitales, but nearly related to the Araucarineae.

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THE PHYLOGENY OF FLOWERING PLANTS[1]

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One might have expected that the publication of the "Origin of Species" about the middle of last century would have had as great an effect on the minds of such distinguished botanists as Bentham, J. D. Hooker, and Asa Gray, as it had on prominent zoölogists and geologists of the same period. We know that Hooker, at any rate, was Darwin's confidant and helper and that "without Hooker's aid Darwin's great work would hardly have been carried out on the botanical side."[2] Yet it remains a fact that the classic "Genera Plantarum" of Bentham and Hooker contains no hint of the descent of plants, begun as it was in the very heyday of "Darwinism."

It was not until the system of Engler and Prantl appeared that we had our first arrangement of the families on supposed phylogenetic lines. Hooker[3] did not share Engler's views as expressed in his classification and writings. He considered Engler and Prantl's System to be "neither better nor worse in the abstract than de Candolle's (so-called), and far more troublesome to apply for practical purposes." He held to Robert Brown's view of the families being reticulately not lineally related.

In comparatively recent years, Engler and Prantl's System has been questioned by several botanists, prominent amongst them being Bessey in America, Hallier in Germany, and Arber and Parkin in Britain. In addition, the German system has received little or no support from palaeontologists. More recently besides the attempt at a rearrangement of the families by Bessey,[4] the author of the present paper has ventured to propose a new system in the "Kew Bulletin" and in a separate book[5] published early this year. On the appearance of the first of these papers in the Kew Bulletin, a prominent American teacher of botany wrote "I have for years put a good deal of thought into teaching a rather old-fashioned course on flowering plants, and I am constantly embarrassed with trying to justify to students the Engler-Prantl System, which is imposed on us by all our recent manuals which the students must use." Others have written to me in the same strain. I notice also that Jepson[6] in California returned to a

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany, joint meeting with section of Taxonomy, Ithaca, New York, Aug. 18, 1926.

[2] Huxley, L. Life and Letters of Sir Joseph Dalton Hooker, **1**: 406. 1918.

[3] Huxley, l.c. **2**: 22.

[4] Bessey, C. E. Annals Missouri Bot. Gard. **2**: 109–164. 1915.

[5] Hutchinson, J. The Families of Flowering Plants, Macmillan Co.: 1926.

[6] Jepson, W. L. Economic Plants of California, 1924.

modification of that of Bentham and Hooker in a small work which he has recently published.

Thus there seems a desire amongst some taxonomists, at any rate of the younger school, and particularly amongst teachers, for a new phylogenetic system, based on more modern ideas of the evolution of the flower, correlated with anatomical structure, growth form, and geographical distribution. It seems desirable that the artificial systems, which were essentially practical and framed mostly for the determination of plants and for little else, should give place to a more natural system, at any rate for teaching purposes, for the deadening effect of these artificial systems, with their dull floral formulas and floral diagrams is only too obvious. The consequence is that many physiologists, ecologists, and palaeontologists of to-day are often severely handicapped for want of knowledge of taxonomic botany.

A few years ago I began a special examination of the Angiosperms with a view to the rearrangement of the families on more logical lines than appeared to be the case in the German System. For this purpose I examined in the great collections at Kew every species and genus of all families which appeared to be likely to furnish evidence of phylogenetic relationships, and at the same time took note of important facts in regard to geographical distribution, regional habitat, anatomical structure and growth-form or habit. It seemed to be quite illogical to entertain the belief that the parts of a flower were derived from leaves, a belief which has never been seriously challenged, and at the same time accept the so-called "Amentiferae" as being the most primitive group of Dicotyledons, as in the Engler and Prantl System. If carpels are really modified leaves, then "polycarpy" and not "syncarpy" would be the primitive condition. We find no polycarpy in the Amentiferae. Then again we should not expect to find in the earliest flowering plants a specialized inflorescence such as we have in the catkin. We should look rather for a solitary terminal flower, as in *Magnolia* or *Ranunculus*.

If we regard the least differentiated type of floral structure as being the most primitive, then we must begin our phylogenetic classification with either the Magnoliaceae or the Ranunculaceae as in the De Candolle System, and as recently so ably advocated by Bessey, Hallier, Arber, Parkin, and others. Our task then is to bridge the gulf between a *Magnolia* and a Composite or Labiate, and at the same time trace the evolution of Monocotyledons. This is no light task, but one which should be the ultimate aim of systematic botany.

Engler regarded the Monocotyledons as the older group, and they precede the Dicotyledons in his system. It should be noted, however, that nearly all the monocotyledonous families are very natural families; that is to say, they are to a large extent homogeneous groups of genera which differ very slightly from one another. I have been led to regard all such natural families as being advanced and most recent in the evolutionary sequence. There is clear evidence of this in the Dicotyledons. Everyone, for instance, considers such families as the Cruciferae, the Malvaceae, the Papilionaceae (Fabaceae), and the Umbelliferae to be advanced and therefore recent families, and they are also the most natural.

As to whether the Monocotyledons are monophyletic or polyphyletic in their origin I do not propose to enter into here, but some of the families, the Alismataceae and Butomaceae, are so closely related to the Ranunculaceae as to suggest a direct origin from the same stock as that family. So that the Monocotyledons appear to have arisen from one of the most primitive groups of the Dicotyledons, and taken as a whole they are the more generally advanced group.

In the more primitive groups of families, at any rate, there seems to be a marked difference in growth form. For example, all the Magnoliaceae are "*woody*," all the Ranunculaceae "*herbaceous*," except some softly woody climbing *Clematis*. In floral structure these two families are very similar, but this similarity is probably due to parallelism, and not to very close affinity, for I have failed to find real "blood-relationship" between any members of Magnoliaceae and of the Ranunculaceae. If they are really related, then the connecting links seem to be lost. Now I do not for a moment suggest that we should go back to the old herbal classification into Trees, Shrubs, and Herbs, for there are of course many familiar examples of families containing both woody and herbaceous plants; but I am convinced that in the more primitive groups, at any rate, the "habit character" is of considerable importance. With it there is associated a very striking difference in the stomata, in the woody groups (Magnoliales for example) the guard cells of the stomata being accompanied by special subsidiary cells parallel to the pore, these being absent from the stomata in the early herbaceous groups (Ranales, Saxifragales, etc.).

The result of all these studies, in more or less skeleton form, is shown in the accompanying diagram (Fig. 1). By means of this diagram or phylogenetic tree I have tried to indicate on broad lines the probable course of evolution of the Dicotyledons. In the majority of the basal groups from which the arrows radiate we find "free" carpels and mostly "numerous" stamens. When syncarpy has been attained, as for instance in Bixales and Rhoeadales, the placentation of the ovules is predominantly parietal, a condition I regard as being on the whole the most primitive type of placentation in the syncarpous ovary, as it is nearly always accompanied by hypogyny and numerous stamens. You will notice that on both sides of the tree at these points families have arisen which have become fixed and successful types. Such are the Passiflorales (Passifloraceae, etc.), Cucurbitales (Curcurbitaceae) on the left; on the right Capparidales (Capparidaceae) and Cruciales (Cruciferae). From these families little or no further evolution has taken place and most of them are very widely distributed. But the culminating stage in the syncarpous ovary appears to be the axile, basal or apical placentation. This condition becomes dominant in such groups as Theales, Tiliales, Rosales, Caryophyllales, etc., and from these groups prolific evolution is evident, accompanied eventually by a great reduction in the number of stamens and ovules, by cohesion of the petals, and by varying degrees of adhesion and modification of the corolla and inflorescence. You will observe that, unlike Bessey, I have retained the Metachlamydeae (Gamopetalae) as a separate group. We must still be a little practical in our system, although the ideal phylogenetic classification will perhaps amalgamate the two groups

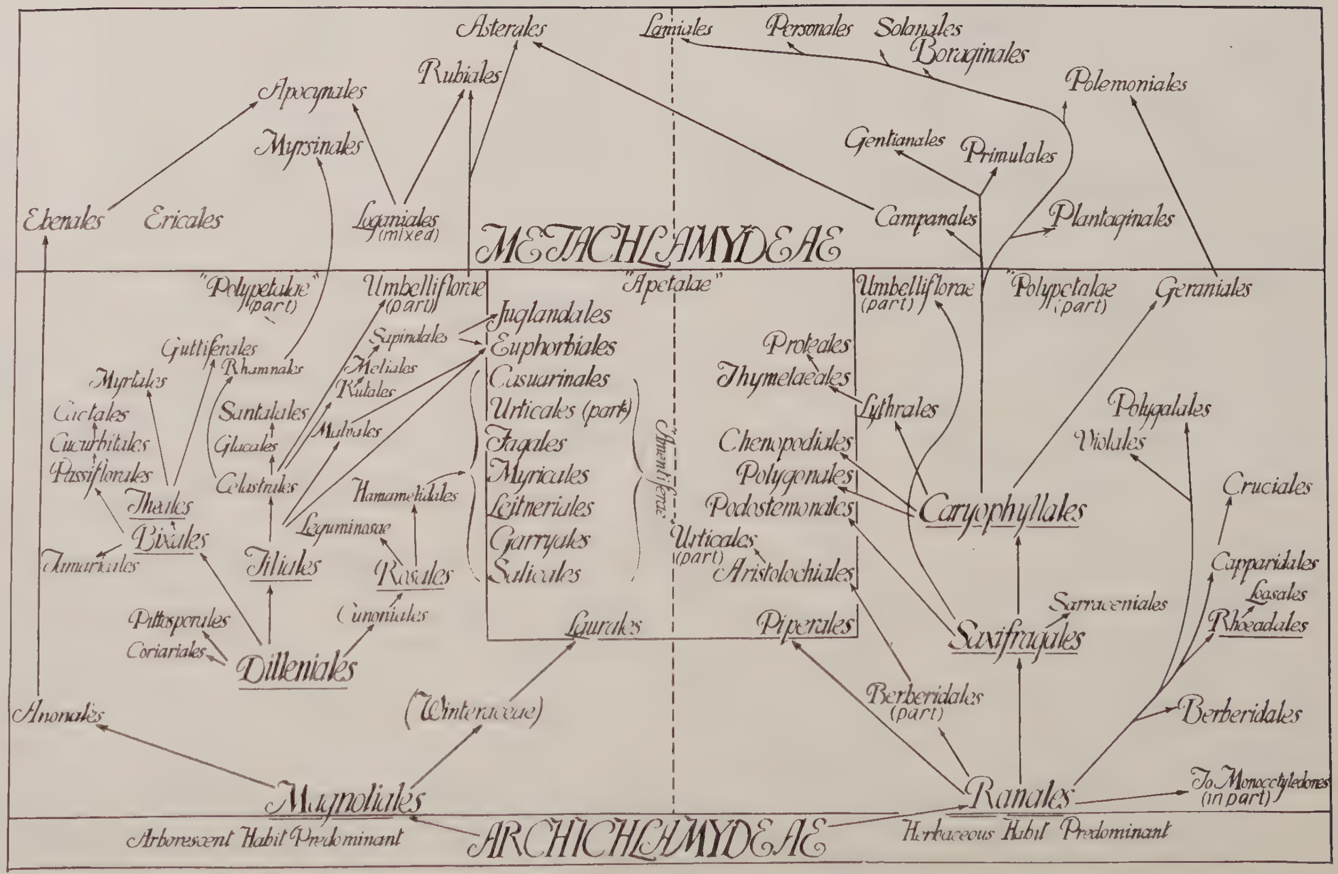

Fig. 1. Probable course of evolution of the Dicotyledons.

Archichlamydeae and Metachlamydeae, depending as they do on a single character, that is, the freedom or cohesion of the petals.

The difficulty of showing in a "linear" sequence the families as arranged in this diagram will be readily appreciated. The best way I could do this was to take groups of families from each half of the diagram and arrange them alternately in sequence, thus bringing together the families showing parallel developments. Below is a page from my recent book which will give an idea of this method. The cross-lines indicate the end of a group, and it is obvious that Family no. 64, Basellaceae is in no way related to Family 65, Linaceae. Basellaceae is the apetalous end of the Carophyllaceous phylum, Linaceae is an advanced and more specialized family supposed to have arisen from the same stock.

PAGE FROM "THE FAMILIES OF FLOWERING PLANTS."[7]

Notes on Affinity (Origin and further development).	Sequence of Orders (Cohorts and Families). A cross line indicates the end of a group.	General Characters and Tendencies of Orders (Cohorts).
	17. Caryophyllales.	
A prolific herbaceous group which has given rise to apetalous hypogynous groups such as Polygonales, Chenopodiales, perigynous petaliferous groups as Lythrales, perigynous apetalous groups as Thymelaeales and part of Metachlamydeae (Gamopetalae).	52. Elatinaceae p. 126 53. Caryophyllaceae, p. 127 54. Molluginaceae p. 128 55. Ficoidaceae (Aizoaceae), p. 129. 56. Portulacaceae, p. 130	Hypogynous to perigynous; ⚥; cyclic; syncarpous; axile to free-central placentation; stamens mostly definite; endosperm copius; embryo curved.
	18. Polygonales.	
Reduced degraded types of Caryophyllales.	57. Polygonaceae, p. 130 58. Illecebraceae, p. 130	More or less as in Caryophyllales but apetalous; ovary 1-celled, 1-ovuled; embryo straight to curved; endosperm copius; stipules often sheathing, intrapetiolar, membranous or scarious.
	19. Chenopodiales	
	59. Phytolaccaceae, p. 132 60. Cynocrambaceae, p. 133 61. Chenopodiaceae, p. 134 62. Batidaceae, p. 134 63. Amarantaceae, p. 135 64. Basellaceae, p. 136.	As in Polygonales, but stipules absent or very small; carpels numerous to solitary, free or connate; embryo curved.
	20. Geraniales.	
Here regarded as advanced more or less fixed types from Caryophyllales; considerable gap between the two groups but affinity evident through Limnanthaceae especially.	65. Linaceae, p. 138 66. Zygophyllaceae, p. 138 67. Geraniaceae, p. 138 68. Limnanthaceae, p. 140 69. Oxalidaceae, p. 140 70. Tropaeolaceae, p. 142 71. Balsaminaceae, p. 143	Hypogynous; ⚥; ovary entire to lobed syncarpous; ovules mostly 1–2 in each cell; stamens definite; disk glands often present; no endosperm; leaves frequently much divided stipulate; higher types have zygomorphic flowers and tendency to syngenesious anthers.

[7] Hutchinson, The families of Flowering Plants, I. Dicotyledons. Macmillan Co.: London, 1926.

In the general sequence I have found it more convenient to place the Asterales befor the Lamiales, partly due to the difficulty of the linear arrangement, but also because I consider that the composite head has been more easily attained than the condition we find so constant a feature of the Labiatae. I think the tendency to form heads or clusters of flowers is much more common throughout nearly all the families than the zygomorphy of the corolla, reduction of the number of the stamens, and gynobasic style which are characteristic of Labiatae. The Labiatae, therefore, might be regarded as the highest expression of the Dicotyledons, equivalents of the Orchids in the Monocotyledons.

Then some comment seems necessary on the position of a few other groups about which there has been controversy. Let us start with the group we all know as "Amentiferae." In the diagram you will notice that I have shown these as having been derived directly from the Rosales through the Hamamelidales. It is perhaps not generally realised that we have already in some Hamamelidaceae inflorescences which approximate very nearly to the catkin. We find there also the bilocular ovary and the characteristic type of leaf so common to many of the "Amentiferae." So we may feel reasonably certain that from a stock somewhat intermediate between Rosaceae and Hamamelidaceae the greater bulk of the "Amentiferae" have arisen.

The Tiliaceae and allied families provide a useful group for the study of phylogeny. It seems very clear that in this group herbs have been derived from the woody forms, the main ultimate development being expressed in the Malvaceae, which are largely herbaceous; but they are herbs with fibrous stems, which seems an indication of evolution from more woody types. The floral structure, of course, bears this out in a very marked degree, especially in the androecium. Then from the largely unisexual Sterculiaceous stock we seem to have the origin of part of the Euphorbiaceae. I say part, because I am convinced after much work on the family that it is polyphyletic. No one could believe that such types as *Hymenocardia*, *Antidesma*, *Hevea*, *Ricinus*, *Euphorbia* and *Croton* have all come from the same stock. Unfortunately the family has an unsuitable name, for the genus *Euphorbia* is practically an exception. The result is that when we speak of Euphorbiaceae we are apt to think only of *Euphorbia*.

Now Engler appears to regard Euphorbiaceae as a product of the Geraniales. In my opinion Euphorbiaceae show little affinity with any of the families composing Engler's Geraniales. I might even go farther and say I fail to find any. A far better place to look for the origin of the family would be the Tiliaceae, Sterculiaceae, Malvaceae, Flacourtiaceae, Celastrales, Olacales, and Sapindales. Perhaps we should even include the Menispermaceae, for has not the greatest living authority on Euphorbiaceae mistaken a common species of *Cocculus* for a new genus[8] of Phyllantheae!

The *Geraniales* of both Bentham and Hooker and of Engler and Prantl are cumbrous groups containing many very widely different families. What is there in common between a *Geranium* and a Mahogany or a *Simaruba*? Perhaps it was the ventral raphe, a mere thread, which bound them together! If we examine

[8] See Flora of Tropical Africa. 6, 1: 441. 1911.

the characters given, we shall find that this is about the only character common to all the families of Geraniales, for it was used to separate them from the Olacales. I fear I have little faith in such a slender character. The ovule might retain its orientation during the course of evolution whilst the other parts of the flower were changing out of all recognition, and conversely it might undergo considerable modification whilst the other floral parts remained stationary. I have now restricted the Geraniales to Linaceae, Zygophyllaceae, Geraniaceae, Limnanthaceae, Oxalidaceae, Tropaeolaceae, and Balsaminaceae. Here we have a natural assemblage of closely allied families with some indications of origin, perhaps rather remote, from the Caryophyllales..

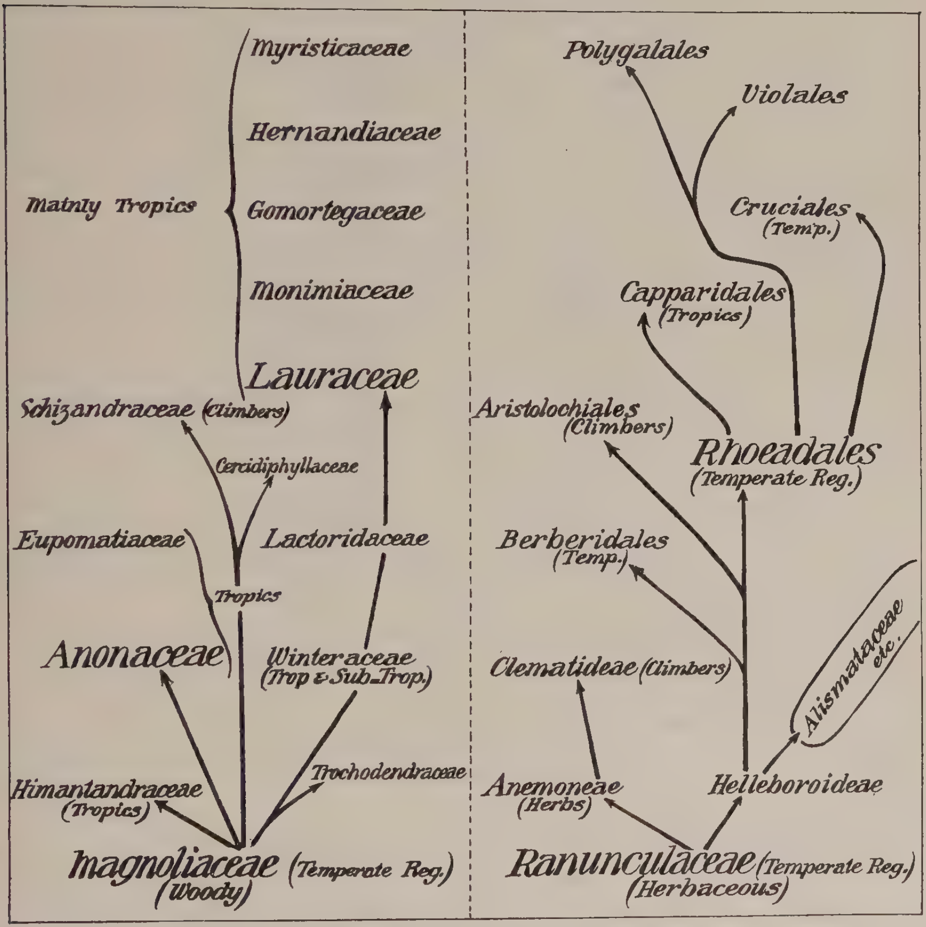

Fig. 2. Phylogenetic relationships of families supposed to be early offshoots from the two basal parallel stocks, Magnoliaceae and Ranunculaceae.

We may now consider briefly the origin of a few other main groups. For instance are Umbelliferae (herbaceous) really related to Araliaceae (woody) or is it due to parallelism? I think the latter. The Umbelliferae seem to have

close connections with Saxifragaceae (herbaceous). In Saxifragaceae we already find the pair of inferior carpels, and some similar tendency to the umbelliform inflorescence. Araliaceae, on the other hand, are probably epigynous representatives of the Celastrales. The Myrtales are in my opinion epigynous representatives of Theales and not closely related to Rosales, as often placed. And before leaving the Archichlamydeae I might mention the problem of the true position of the Proteaceae, that peculiar family almost exclusively confined to Southern Africa and Australia. Now the Australian flora on the whole is not an ancient flora. It is rich in large natural and comparatively recent families such as Myrtaceae, Papilionaceae, Mimosaceae, Epacridaceae, Compositae, and Gramineae. Nor are there many ancient families represented in Southern Africa. A large proportion of the vegetation in these two areas consists of Proteaceae, and I consider Proteaceae from its floral structure to be quite recent. Probably it had its origin in the Southern Hemisphere and from the same stock as the Thymelaeaceae. The inflorescence of such a genus as *Protea* is almost equivalent to that of a Composite, whilst that of *Leucadendron* is cone-like after the manner of *Alnus*.

In regard to Metachlamydeae or Gamopetalae I have not much to say of an original nature. The origin of the Ericales from Theales, and the Gentianales and Primulales from Caryophyllales is now generally admitted. The Compositae I consider to be somewhat polyphyletic. It seems probable that the subfamily Cichorieae, with their milky juice and homogamous heads, have not had quite the same origin as the Helianthoideae or the Vernonieae. Of course, Professor James Small of Belfast has told us that by a sudden mutation the genus *Senecio* arose on the South American Andes, at a time, as Professor Berry has since pointed out, when there were neither mountains nor even land in that region. So that the genus from which he thought it might have sprung, *Siphocampylus* could not possibly have existed. We must look for the most primitive Composite, not in *Senecio*, to my mind one of the most recent genera, but in the tribe Helianthoideae, which has many primitive features.

Loganiaceae is a mixed family which seems to show the origin of both Apocynaceae and Rubiaceae. It is interesting to note that the almost entirely woody family Rubiaceae has retained during the long course of its evolution the stomata characteristic of the Magnoliales. There remains only the long series of families beginning with the Polemoniales and ending with the Labiatae, which find their origin, I think, in the geranial stock, and which on the whole seem to be the highest expression of evolution in the Dicotyledons.

In Figure 2 I have shown how a few of the primitive families may be arranged, having regard to their floral characters, general anatomical structure, and growth forms. In these groups especially there seems something to be learned from their regional distribution. This is a marked tendency for the basal or more primitive groups to be dominant in the Temperate Regions, the more advanced or reduced groups to be dominant in the Tropical Regions, as if the course of evolution had at first been from the cool to the warmer parts where the types soon became fixed. This is well shown by the distribution of Magnoliaceae

(ancient and temperate) and Anonaceae (modern and tropical) respectively, and by the Ranunculaceae and Piperaceae.

I should also emphasize the fact that it is not suggested that the families of flowering plants have arisen one from another as they now exist. For instance in indicating that the Fumariaceae are believed to have arisen from the Papaveraceae, I mean that they arose from the same basal stock, which was probably quite tentative and soon resulted in the advanced types which we know at the present day.

Fig. 3. *Magnolia pterocarpa* Roxb., a species from the Himalaya, regarded as the most ancient living angiosperm; in A note the diminishing upper leaves, and in B and C the foliaceous tips of the carpels.

Finally, in Figure 3, I give an illustration of a species of *Magnolia* (*M. pterocarpa* Roxb. from the Himalaya) which I regard as being the most ancient living Angiosperm, in fact like *Ginkgo*, almost "a living fossil." In this plant the upper leaves (Fig. 3, A) are separated by very short internodes and they gradually decrease in size towards the sepals and petals. The carpels (Fig. 3, C) have also very striking leaf-like tips, and this species seems to show that the theories now put forward are at any rate reasonable probabilities.

THE RÔLE OF FLOWER ANATOMY IN THE DETERMINATION OF ANGIOSPERM PHYLOGENY[1]

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The natural classification of plants is based in considerable part upon the slowness to change of method of reproduction and of structure of reproductive organs. Experience has shown that such a basis for the determination of phylogenetic relationships, in its broader aspects at least, is sound. Other aspects of structure and behavior have been used as accessory evidence of relationship. Among the higher plants the structure of the vascular skeleton has in recent years been increasingly emphasized as also slow to change during evolutionary modification and hence as of much value in phylogenetic studies. This conservatism of vascular structure was long ago noted and has been generally recognized as important in the classification of the larger groups of plants. Of late it has become more prominent chiefly because of the development and substantiation of the stelar theory. Certain features of vascular structure have to some extent been made use of by taxonomists as additional evidence of relationship, or have raised questions of relationship which might otherwise not have arisen.

The skeleton of plants is in some respects—among these, conservatism in change—like the skeleton of animals. And it is indeed remarkable that the plant skeleton has been comparatively so little studied for evidence of ancestral structure and hence of relationship. Among animals the skeletal structure is of very great importance in studies in comparative morphology and phylogeny. Not only does such study give evidence of direct evolutionary modification, that is, elaboration of parts and fusion of parts, but it presents particularly convincing evidence of reduction and of loss of organs,—for example, the presence in certain reptiles and mammals of vestigial skeletal parts for appendages which have been reduced in evolution and are no longer present as externally recognizable structures. In the plant skeleton closely parallel conditions obtain.

Morphologists have again and again called attention to the value of vascular structure as indicating relationship among the larger groups of vascular plants, but the value of the anatomy of the skeleton for the determination of relationships within small groups has received very little attention. It is, of course, true that the differences between small groups are not so readily recognized and require more detailed study for their determination. The determination of relationship among the smaller groups of plants must be based upon the lesser features of vascular structure, and since in such groups it is in the flower that

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany, joint meeting with section of Taxonomy, Ithaca, New York, Aug. 18, 1926.

differences of critical importance have been recognized, so it is there that anatomical evidence, at least in part, should also be looked for.

The most important evolutionary changes in the structure of the flower are (1) those involving the fusion of parts and their consequent greater or less submersion as individual parts in the united mass; and (2) those of reduction, either attendant upon cohesion and adnation, or due to definite, direct loss as individual organs. Such changes may be evidenced more or less clearly by external features (especially under comparative study); but they can also be demonstrated by the structure of the vascular skeleton, and more clearly by the latter since this is slower to change than is the external configuration of the organs. For example; where organs become fused phylogenetically, as stamens with petals, proof that the condition of epipetaly of the stamen is merely one of adnation may not be evident externally, but internally there is clear proof in the presence of independent vascular supplies to the two organs. Where organs have been lost phylogenetically, no remnant may be present externally, but the "stub" of the vascular supply may remain and constitute proof of the former existence of the organ.

In such ways as these there is often preserved in the vascular structure of a flower for some time after external change has occurred, a record of the structural condition possessed by ancestral forms. Thus, from the skeleton there can often be obtained evidence of descent where other proof may be wholly lacking. In this way evidence of the probable relationships of many groups of living forms may be obtained.

This method is especially adaptable to cases of doubtful structure and to those of suspected or certain reduction. Naturally the flower-forms most difficult of interpretation are those in which through the loss of parts the flower has become simple. If vascular anatomy can demonstrate the true morphological nature of a complex flower, and whether a simple flower is simple because of the possession of primitive structure or because of reduction, important progress can be made toward an accurate determination of phylogeny. If, for example, the flowers of *Salix* can be shown to be really modified from complex flowers and that they are not simple, primitive flowers, opinion as to the relationships of the willows is very different from that generally accepted today,—that these forms are among the most primitive living angiosperms. If the plan of the skeleton found in the flowers of the Caprifoliaceae is the same as that found in the Cornaceae, perhaps these families are more closely related than they are commonly assumed to be, and evidence from inflorescence-form, wood-structure, etc., as to close relationship is reinforced.

Briefly, then, the anatomy of the flower will help in the solution of obscure structure and of morphological puzzles and will give proof of the existence in ancestral forms of organs now lost. This is because of the so-called conservatism of the vascular skeleton; and because in the skeleton of the flower there is still greater conservatism since there is here combined the slowness to change of reproductive structures and that of the skeleton.

The following examples may aid in making these points clear. The classic example of the anatomical method in the solution of obscure structure is that of the orchid flower. No one questions today the nature of the "column" of the orchid flower, or wonders that anthers should be borne, apparently, on the style. The study of the course of the bundles supplying the stamens and the stigma definitely demonstrated 75 years ago that the stamens are adnate to the style and that one or two of them are reduced. So convincing is the external evidence of this that it hardly seems today as though suggestions from anatomy could have been necessary in the beginning.

As an example of another type of obscure structure, the nature of the ovary in certain genera of the Caprifoliaceae may be taken. Here, there is no question, from external evidence alone, but that there is fusion and reduction of a primitive 5-carpel, 5-chambered condition; but the structure of the more reduced genera is determined completely, and with certainty, only by anatomical study. In *Sambucus* there appear to be but three carpels, yet the vascular supply for two other carpels is present. In *Viburnum* there is a unilocular overy with 1-3 stigmas and about five ovules, all but one of which are abnormal. On anatomical evidence it is clear there are three well-defined carpels present, and evidence of two others is present in some cases. The three well-developed carpels are so fused and modified that there is one loculus below and three above. There are three placentae, only one of which bears a normal ovule.

The question as to whether basal ovules are "cauline" or "carpellary" may, at least often, be answered by the determination of the course of the bundle supply to the ovule. In *Boehmeria*, for example, the bundle to the ovule passes not directly into the ovule from the carpellary supply, but ascends the ovary wall for a short distance, then turns abruptly and descends to the base of the ovule. This peculiar course surely indicates that the ovule has descended from an original lateral position—a condition found in allied genera.

Anatomical proof of lost organs ranges in kind from that which merely corroborates evidence otherwise obtained to that which supplies entirely new facts.

That the achene of the Ranunculaceae with its solitary ovule has been derived from a follicle with several ovules has long been generally accepted on comparative evidence. Further proof exists anatomically in that in intermediate forms vestigial vascular bundles lead to the position of the lost ovules.

Within the Primulaceae there has clearly occurred the loss of a whorl of stamens. *Lysimachia* alone would provide proof of this, since five vestigial vascular bundles run to the position of the five lost stamens. Similarly, within the Ericaceae ten carpels have been reduced to five: *Gaylussacia*, on anatomical evidence, clearly has ten, *Vaccinium* has five and five vestigial ones; other genera have five with no trace of more.

In *Carex* the primitive members of the genus retain the rachilla within the perigynium; more advanced species have lost this spikelet-axis and the flower is apparently terminal. Proof that the flower is really lateral, however, and that the axis formerly extended beyond the flower, is to be found in the presence of a vestigial vascular stele fused laterally to the base of the ovary.

In the Salicaceae it is clear from the vascular structure that the flower formerly possessed a highly developed perianth, doubtless of two whorls. The nectaries of *Salix* represent morphologically some of the lost perianth parts. It is also evident that the inflorescence has been derived from a more complex type.

Other simple flowers have also been similarly shown to be reduced. The flowers of *Ulmus*, for example, have been derived from forms with another whorl of perianth parts and another whorl of stamens. The 2-carpel pistil shows vestigial evidence of several additional carpels. Many similar examples may be cited.

The anatomy of the flower is as yet little understood. It is, however, by no means an untouched field. Van Tieghem, an exponent of the anatomical basis in all phases of morphological study himself worked rather extensively on the flower, especially the pistil, and Henslow delt at length with flower anatomy in his studies on the evolution of floral structure. These investigators recognized the principle of fusion of the skeleton under cohesion and adnation and also that of the persistence of vestigial bundles. However, neither by them nor by their followers have these principles been applied to any extent in the determination of phylogeny. Further, an understanding of basic flower structure can only be had when a large and representative lot of plants has been studied with much care. This has not as yet been done. Still further, the study of a particular group must be carried through in great detail if the anatomy of the flower of that group is to be known, and particularly if the question of lost organs is involved. This is necessary because some species of a genus may show no evidence of lost organs and other species may present this evidence very clearly.

It is apparent that there is an immense amount of work to be done and that only very gradually can a new system based in part on anatomical evidence be built up. Hence at the present time rather few questions of relationship among angiosperms can be answered from an anatomical basis. However, general replies can be made to some of the more important questions which arise in the establishment of a more closely natural system, for example, the following:

In recent years much dissatisfaction has arisen with the Engler system as an expression of natural relationships. This system is the only one, claimed to be natural, that has been widely used. It is based upon a series of principles most of which are not called in question at present. One of the most important of these principles, however,—that simple, naked flowers are primitive,—seems to arouse most doubt as to its validity in the minds of taxonomists and morphologists alike. Much evidence, of varied nature, points to the fact that many, at least, of the forms with simple flowers—which are placed at the base of the Engler system—are reduced. If this is so, such forms should not be placed in this part of the system, and the phylogenetic tree will be markedly changed when such groups are given a natural position.

The question of whether simple flowers are primitive or reduced is obviously one of fundamental importance to the establishment of a system of classification that shall be as closely natural as possible. Simple flowers have of course been variously treated. Some, such as those of the ash, are looked upon, apparently

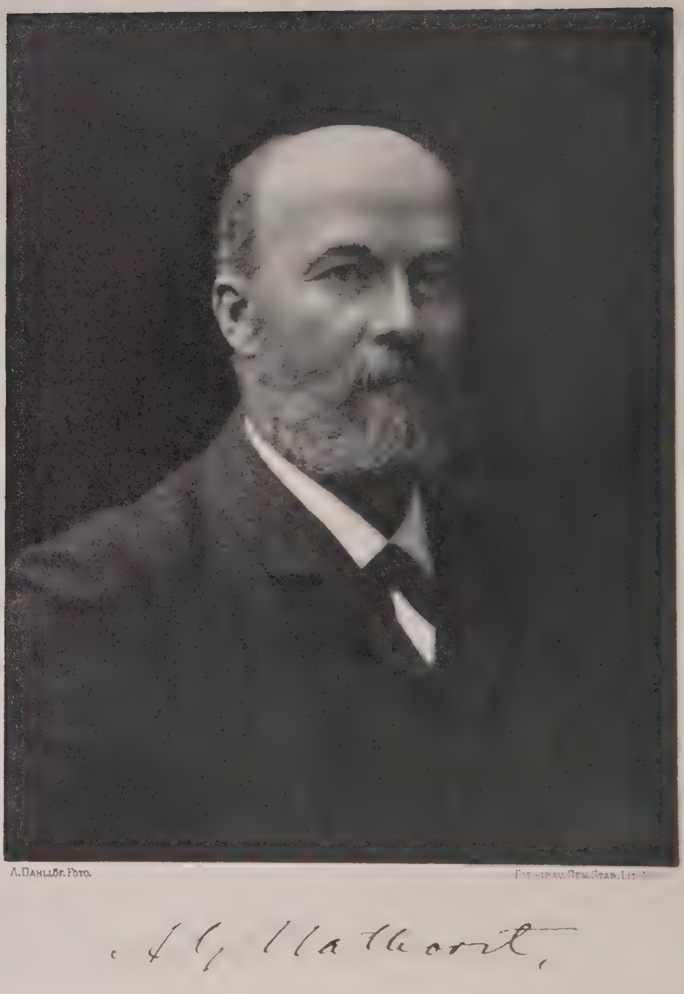
generally, as reduced; others, such as those of the Amentiferae, are often called in question. Anatomy, together with comparative morphology, has shown that many of the Amentiferae are certainly not primitive; the structure of their flowers shows that the apparently simple condition is a derived one, the ancestral forms having had more complex flowers.

All simple flowers must be tested in every way to determine whether they are actually primitively simple. Anatomical evidence should be of the greatest value in these determinations. It should similarly have much weight in those interpretations of flower structure which determine more directly the relationship of supposedly "close" families.

Anatomists do not of course make the claim that anatomical study will in all cases solve questions of relationship; but they do believe that here is a greatly neglected field from which evidence may very often be obtained,—and they believe that such evidence is of the strongest. It is true, of course, that such evidence as the presence of vestigial bundles (indicating the former existence of organs now lost) may not always be found even when apparently most likely to occur. A corolla may have apparently been lost rather recently, as in the ashes, yet there may be no evidence as to its former presence. When such evidence is lacking—and it should always be sought—this lack is not, of course, proof that the suspected organs may not have existed. Often the vascular traces disappear with the organ, and not infrequently even before the organ. Vestigial organs may thus be without vascular supply.

Before definite conclusions can be drawn as to the phyletic status of any plant, evidence must be in from all fields, and all must be weighed. Anatomy should aid in the supply of testimony.

The field in which detailed studies of flower morphology can be made from the standpoint of phylogeny is very large. There is need of a great deal of study before a basis of interpretation can be firmly established. Many taxonomists are interested in the development of a new system of classification which shall be more accurately natural than that of Engler. Will not anatomists and morphologists render assistance in this field and work with taxonomists toward the completion of a system of angiosperm phylogeny which shall be as natural as possible, and thereby render a service to all students of biology?



A. G. Nathorst, 1850–1921.

ANTIQUITY OF THE ANGIOSPERMS[1]

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Solms and Scott have both stressed the fact that in studying fossil plants the great aim is "the completion of the natural system." Indeed there can scarcely be two opinions here. The natural system may never be completed. Parallelism and homoplasy, using the excellent term of Cope for the origin of similar structures in separate stocks, may have been so intense throughout geologic time, lines of descent so old, and perhaps transmigrations from the sea so varied and prolonged, that the actual, the special courses of change leading into the present world must always remain obscure. None the less, botanical science has passed far the point where any doubt can remain about either the objects or the methods of plant investigation. The dynamic value of coördinated study of the present and the ancient plants is sensed by every botanist. The time is even near when fossil material will be conveniently drawn upon in elementary courses in botany; while the need of a bettered chronology is the ever present one that goes back to the time of Cuvier and Brongniart.

In venturing to join in the present discussions, I am mindful of my lack of botanical training—never severe enough to give me critical views of angiosperm taxonomy sufficiently tested from data of structure. Were the subject the history of the gymnosperms I should be more confident. There the facts added during my own thirty years of contact with the steadily extending record seem to hold transcendent interest. The noble group Cordaitales may have as one of its progenitors the *Paleopitys* of the mid-Devonian, and it ends, as I had the fortune to find, in the *Noeggerathiopsis* of the Mexican Lias. The seed-bearing quasi ferns are a marvelously well known group, the discovery of which was fairly predicted by Stur—in a definite way too, by myself. "Too much like ferns to actually be ferns, unless down about the *Psilophyton* level," as Scott suggests, no ancient group of plants more profoundly affects our conceptions of gymnosperm evolution. But that ancient group which has proven as surprising as the seed ferns, the discovery of which began nearly a hundred years ago, and which promises a certain possibility of wide contacts with modern plants, is surely the cycadeoids. Of the eight great groups of gymnosperms, three are wholly extinct, the Cordaitales, the Cycadofilicales, and the cycadeoids or Hemicycadales. But the history of all eight has so lengthened out that it can now be seen that there has been little post-Permian contact between any of the five persisting groups. The presence of typical Araucarians in the Rhaetic will have to be disposed of

[1] Presented before the International Congress of Plant Sciences, Section of Morphology Histology, and Paleobotany, joint meeting with Section of Taxonomy, Ithaca, New York, Aug. 18, 1926.

before this statement need be much modified. Only in the case of the Gnetaleans does the ancient background seem to fail, or is as evidently the fact, hidden amongst the older lignites. As Berry indicates, there may be an undetermined Potomac leaf record; though as Seward suggests, the Gnetaleans may be merely a blind line paralleling the angiosperms. It does seem that the Gnetaleans, though presenting remarkable approximations to the angiosperms, and actually reaching the most utterly primitive floral structure known, failed to advance much beyond the stage of the persisting forms.

However, the extinct gymnosperms already greatly outnumber the existing species, and that much more is not already known is merely due to fewness of paleobotanists, and a course of field work still far more dependent on chance than in the two other branches of fossil study, the invertebrates and vertebrates.

The Angiosperm Problem

Contrasted with the gymnosperms, the isolation of the angiosperms is amazing! The phytologic catalogue seems to indicate the presence of over 100 families of Cretaceous-Tertiary angiospermous leaf types, and the general record now seems to extend back to the Rhaetic or transition beds from Trias to Jura. Yet from this great stretch of time, and from amongst all these thousands of fossil forms, there comes no consecutive evidence for the course of change ending in the flowering plants. The field work of many years must be done before some of the closer contacts with the precedent groups can be surely discerned. But the larger outlines of that worldwide course of change that led into the dominant forests of today are fast coming into view; and it is that phase of our subject that it is here wished to discuss. Geologists are fond of reminding botanists of that celebrated remark of Charles Darwin, made in 1879 about the "abominable mystery" of angiosperm origin; and there are those who to this hour think the mystery still hangs low. Let such, be they geologists or not, confine their attention to the *stratigraphic hornbook*. That "mystery" must be mainly resolved by botanists; nor is it more a mystery tonight than is the origin of the birds, or of the mammals.

That geologic time is very much longer than earlier supposed greatly modifies present views of plant evolution. It is seen that progressive change has been more general and also infinitely slower than once believed. That is, plant life is seen to be more resistant to environmental change, origins less local. While much of the visible change bears more on the subject of extinction than on that of origin. Hence the fossil plant record as at present known is more broken than has been admitted in most discussion. As a consequence, each time the earliest occurrence of species and genera finds note, the point stressed is distribution—not origin.

Nevertheless, all this is not so fatal. There are many points at which the systematist and the morphologist are released from earlier extreme limitations. The chronology of what is known has become a subject of importance, one worthy of the closest examination. And too, the significance of the comparative morphology, taking floral, seed, and stem structures alike, is reaching clarity.

The manner in which the fossil evidence bulks up, numerical relations, and the probable numbers of extinct species, why some types are abundant and others fail, data of climatic conditions, the method of inferences derived from plant association and succession, the prevailing phytologic cast from age to age, these are some of the byways leading into the greater field of inquiry.

Specialized Versus Dominant Fossil Forests

Conifers reach specialization in the Trias, and from then until the dicotyls thrust them aside about mid-Cretaceous time are imposing forest-makers. One of the greatest of all forests is the circum-Arctic redwood forest visible during much of Cretaceous and Tertiary time. Yet, the coniferous element in the fossil floras must not be taken too quantitatively. Exactly that specialization led to relatively easy fossilization. Moreover, many of the more leafy conifers must have had the ability to live in mixed forests which left few traces, so that a large part of the conifer record may not denote pure stands at all. In fact, the ancient types of pure stand forests, and the ecology of the conifers and cycadeoids of the Mesophyte or forest canopy of Triaso-Jurassic times, are subjects of the future. It is merely seen that in Jurassic time the Cenophyte with the early dicot vegetation it certainly then contained, finally supplanted the various left-over types of the Paleophyte, and most of the Mesophyte.

Older Angiosperm Stem Fossilization

Conversly to the manner in which the fossil data so far assembled bulks up, it is believed from all the lines of evidence and approach here considered, that the pre-Cretaceous angiosperms may well have been more abundant than the contemporary conifers. But the strangest point of all is that the stems have proven so utterly rare and difficult to discern. The oldest known examples of petrified stems are the four well preserved genera from the English Greensand, excellently described by Dr. Marie Stopes. The types have in part rather small vessels, but are quite without features decidedly primitive. The post-Cretaceous silicified stems are less rare objects, such stems of palms at times occurring in even a striking abundance and beauty of mineralization. Silicification occurs as a rule, although fine calcification is also occasional.

Relatively to conifer stems, however, the chance that an angiosperm stem might be petrified has long been slight; and as neither type of stem has ever been petrified in laboratories, the fuller reason for this curious fact is not understood. Evidently, the oily and resinous content, taken with the porosity and parenchymatous features of given stems, controls the course, and affects the durability of lignitization; and while siliceous infiltration may be the first step toward petrifaction, many examples of partly lignitized, or carbonized and partly silicified stems have been seen in the field, both in superficial deposits, and from the deeper strata in mines. These latter may be partly impregnated by soluble salts.

The actual angiosperm stem record of importance is lignitic, and yet to be investigated by the nitro-cellulose method. This is also true of the floral record!

The older lignites as yet appear to have more scientific than economic importance.

Rarity of Fossil Flowers

The chance for the fossilization of angiospermous flowers in a form recognizable by the cruder methods of fossil plant study is seen to be about negligible. Those flowers could be abundant, both large and small, and cosmopolitan all the way back to Permian times, without leaving any better marked record than has been so far brought to light. In reality it is probable that floral structures have been mainly inconspicuous far back into the Mesozoic, and that the greater cones of cycads, of pines and araucarians, the larger flowers of the cycadeoids and of the magnolias, are all alike examples of gigantism.

Ordinary arithmetic, however, explains the absence of flowers as compared with cones and seeds in the older angiosperm record. The recent and as yet lone discovery of the tiny fruits with the stamens of the Caytoniales first in the Lias of the Yorkshire coast, and then in the Rhaetic of Greenland is about what should be expected. Improved methods of study like those used by Thomas ('25) in demonstrating the Caytoniales can alone suffice to bring into view the older floral structures. The methods also of Nathorst, and of Jeffrey are indispensable in the case of other unusual forms of conservation. Fossil imprints of flowers with features discernible to the eye or when placed directly under the lens, must always remain exceedingly rare. Such, like the Miocene *Combretanthites* of Berry and some other citable forms, do occur; but their increasing rarity, going back in time, shows that compared with other types of fossils they are impermanent objects. Moreover, the generalized antecedent plant types must in all time have lived in the continental interiors, over the uplands, and along the mountain ranges, or exactly in those situations where fossilization in any form was least likely to occur. There are here no facts of significance negating a long angiospermous floral record stretching back to the Permian and even the Carboniferous. It need surprise no one if the monocot similitude pointed out in the stems of the Illinois coal balls by Noé and by Hoskins went with simple floral, rather than strobilar structures. (Wieland, '24).

The Angiosperm Leaf Record

On turning to the leaf record, more conveniently mentioned third, there is again no evidence favoring the validity of the older view that the failure to recognize dicotyl elements in pre-Cretaceous rocks means the absence of such in fact. Though here the case is different. If the characteristic angiosperms of the lower Cretaceous had any ancestry at all, unlike the flowers, the leaves of those ancestral forms had an average chance for fossilization. Is there any reason to suppose from fossil leaves, then, that there was a greatly limited ancestry, that is, limited in both time and geographically, with excessive change in a monophyletic stock? There is none whatever. On the contrary, it is evident that the leaf changes, coördinated in some transpiratory manner with the going out of the dinosaurs and the rise of the mammals and the birds, came last, formed

the last great structural link in the rise of the angiosperms. The hypothetical transition types are abundant. In an earlier address on climate[2] I have stressed the importance of the simple-bladed elements of the Taeniopterid type in the older Mesozoic floras, and outlined the manner in which a marginal net would arise. The "argument" is a very simple one, and there is at least no trouble to find consecutive evidence to fit it. In addition, Bailey and Sinnott ('15) have shown that palmate types may be quite primitive, and I think it instructive to note in that connection the three parallel veins of the young cotyledons of the maple. If moreover the Caytoniales actually had the *Sagenopteris* type of leaf, a third very simple and great course of leaf change from types abundant throughout the Mesozoic, into modern dicotyl nets appears to view.

It is assumed primarily that the main mass of lower Mesozoic angiospermous foliage is as yet obscured in the great leaf complex mainly recorded under the generic names *Oleandridium*, *Taeniopteris*, *Sagenopteris*, *Glossopteris*, *Stangerites* and the cycadeoid *Nilssonia*. These are all genera of convenience, without clearly defined limits, and of world-wide occurrence from the Permian down. They are far more conspicuous elements amongst the plant types as seen in the field than can be understood from lists of record-species alone. They are partly pteridophytic, but are found more and more to include diverse elements,—amongst the Nilssonias and forms with *Stangeria* venation, running up into the flowering plants. Moreover it is just this older aggregate of bladed types that recedes from view as angiospermous foliage becomes conspicuous. In the blade theory of the leaf, the *Ficus*, the dogbane, and the magnolia appear to best illustrate the conspicuous points of change from pro-angiospermous foliage. But it is quite certain that palmate or trident forms like the maple and the sassafras also have their points of contact with older types of foliage. It took from Rhaetic to Dakota Cretaceous time for the developmeet of the typical dicot net canopy.

That the direct course of change suggested is possible and probable, and that it was at its peak in the Jura finds a certain sharp reflection in the lower Potomac floras of Maryland. Berry says, "With regard to the ovate forms of *Protæephyllum* with a midrib, these may be angiospermous, or they may be related to *Ficophyllum* and *Rogersia*, which may be early representatives of the Gnetales." The irregular subrhombic type of net in these leaves is also seen in the presumed cycadeoids. Thus Fontaine considered the very characteristic *Acaciæphyllum* to be an angiosperm, though one sees that Berry is correct in referring it to *Thinnfeldia*.

The point should be made emphatic that in arguing older angiosperm points of contact with cycadeoids the type of incipient netting seen in *Ctenis* and *Dictyozamites* can merely be cited as indicative of the widespread tendency toward netted venation evident in very diverse Jurassic and lower cretaceous plant types. The netted cycadeoids so far discovered were doubtless quite as highly specialized as *Cycadeoidea* itself, and perhaps as remote from the angiosperms as modern

[2] Wieland, G. R. Triasso-Jurassic Plant Evolution and Climate. American Naturalist, **19**: 452–474. Sept.–Oct. 1925.

cycads. I would like to add here that the vegetative likeness between Cycadeodeans and recent cycads is even more complete than first supposed. From the sections of the *Raumeria* of Dresden just completed, it is found that the cycadeoids also show the transition from the old scalariform wood into the pit wood. This parallelism between the two groups is a cardinal point of reference for students of stem and foliar structure, and especially for ecologists.

Paleophytology has long been, and must in any broader view remain the foundation on which the knowledge of ancient floras must rest. But too much can be made of the gaps in the foliar record. Those subtler changes in bundle and stem structure must represent greater evolutionary steps than most openly observable foliar changes. At first sight the gap between a pinnately veined blade of some early cycad and a bipinnate frond like that of *Bowenia* appears all but impossible to cross. And yet that change was accomplished in a very simple manner. Abnormal fronds of *Stangeria* show the manner in which the change from the pinnate to the bipinnate condition sets in. It is seen that the entire change could become fixed almost instanteously measured in units of geologic time. Moreover, the manner of change does not fail of analogy to changes from the simpler to the compound net veined types, except that in them the course of change further depends on the extent and form of the vein islets. That which should astonish most in viewing the older leaf record is, after all, the definitiveness of floras and of the larger courses of change. It is amazing to see the origin perhaps widely separated in time and geographically too, of so exactly the same type of xerophyllous frond in such far separated types as the cycadeoids of the Black Hills and the Galician Carpathians, in the first instance, and of the present-day cycads of Mexico and Australia, in the second. But the wide occurrence in mid-Cretaceous rocks of Magnolias and *Liriodendropsis* is in a sense a sequel.

Mesozoic Trend of Change

So far as any consensus of the facts of fossil plant occurrence and those more directly indicated trends of change may aid, what is the main conclusion to be drawn with respect to type and abundance of the older angiosperms? Perhaps this: During Jurassic times when pines gradually became specialized in leaf and cone, the angiosperms reached great leaf, floral, and stem variation. In that sense angiosperms are recent. But there is in the main every reason to view them, so far as actual descent goes, as old, just as old as pines, and polyphyletic. That is the only conclusion admissible when the general facts of the fossil record are considered in connection with such casual discovery as can be cited. The reasons why the actual angiosperm record is so meager have been partly given. But the key discoveries will be forthcoming. Meanwhile, it has become evident that there was a great race of flowering gymnosperms, many belonging to the "Williamsonian tribe," many yet utterly unknown. From somewhere within or near this plexus, the angiosperms arose.

The Flowering Gymnosperms

That the carpellary crown of *Cycas* must in some manner foreshadow the grouped carpels of angiospermous flowers, is an old idea of botanists. That, too,

there was some ancient advance from open strobili of cycads, cordaites or conifers into gymnospermous cones, and thence into angiospermous flowers, is another old idea. But such ideas could hardly reach clarity so long as that singular *Tumboa* disk with its obsolete ovule remained the only citable instance of a gymnospermous flower from either the ancient or the modern world.

Down to the year 1899 no fossil fruits or flowers were known to indicate either the direct evolution of the dicotyls or their derivation secondarily from within the gymnosperm stocks. Then came the discovery of the staminate disk of *Cycadeoidea ingens* of the Black Hills. As it happened, the first event in the petrifaction of the great columnar stem which bore the disk occurred just as the stamens approached maturity, and while the central cone was still immature,—a condition which led to fine outlines of the flower-bud with its incurved stamens, but to the loss of nearly the entire central cone. A very small area of the periphery of the cone was noted; but owing to chalcedonization, lightness of color, and strangeness of feature the structure was not understood. The axis, it seemed at the moment the longitudinal and transverse surfaces were first polished and confronted, might have been proliferate; or it might have borne a central group of carpels, an apical seed cone, or even a single seed. *Cordaianthus Penjoni*, also with an unfossilized apex or center which might have been ovulate, was recalled; but the Cordaite stamens seemed by comparison strangely simple. Obviously the stamens were of Marattiaceous form, although their emplacement was on a floral, instead of any anticipated strobilar plan. The features were ancient, but the actual stage of development stood far in advance of anything comparable in existing cycads. As then stated, it was scarcely to have been expected that types bearing such strong testimony to the correctness of the older views of a fern-cycad ancestry should display such a marked combination of advanced as well as ancestral characters. The seed ferns were then unknown.

Nearly complete flowers of the splendid stems of *Cycadeoidea dacotensis* were next cut, and in the account of the floral organization given in 1901, comparison with the flower of the "tulip tree" was emphasized. But a difficulty still stood in the way of such comparisons, in reality more justifiable than was at once evident. The summits of the *Cycadeoidea* buds were usually much eroded or broken off, so that it was not at once seen that there was in addition to the evenly incurved deflexed stamens, a symmetrical dome-like termination formed by an outer petaloid disk to which the stamens were fused. Nor was the fact at once observed that here and there amongst the cycadeoids the inner bracts may vary from the outer ones and thus become sepaloid or petaloid. That the double bundle ring of the lower transverse sections of the campanula did not arise from the bundle patterns of the fused stamens alone, but from both stamens and petals, was not at once so obvious as it may now seem. Note the illustration of these structures given on Plates 1, 2.

While the general comparison of the new anthostrobilar flowers with those of the "tulip tree" was precise, and while the double bundle ring and campanulate form suggested ('09) that sympetalous angiospermous types could have appeared very early, theory could at first be pushed but little further. Relationship of the

cycadeoids to the cycads was proven to be merely vegetative; contact with other gymnosperms was remote, and no actual lines of descent leading towards the Magnoliaceæ were as yet visible. Especially the seed cones remained as they had proven at the time of their first discovery in 1870, considerable of an enigma. Were the stems primitive, or were they reduced? Who might say? *Cycadeoidea* being such a specialized type in all but the stamens and emplacement of the floral organs, any closely defined relationships to other flowering gymnosperms still in the earlier carpellate stage, or to yet other types vegetatively more angiospermous, could not be visualized.

Some new and distinctive discovery here became a *sine qua non*. This was soon made. A. G. Nathorst ('02, '09), immediately following the first descriptions of the *Cycadeoidea* flower-buds, began examining anew the slender forked stems, and small Williamsonia-like cones, found partly carbonized, or as casts or imprints closely associated with the leaves called *Anomozamites*, in the Rhaetic strata of southern Sweden. In the study of 1902, it was proven in brilliant manner that the stems, leaves, and cones belonged to one and the same plant, a partial restoration (Pl. 3) being given under the name *Williamsonia angustifolia*. Here at last, was a type with cones nearly like those of the cycadeoids, small, narrow bladed leaves typically microphyllous, and branched stems with peduncles, cones, or fruits borne in the forks in a manner closely simulating that of magnolias! (Compare Pl. 5.)

If, as a specialized plant *Cycadeoidea* had pointed toward a hidden, unsuspected race of flowering gymnosperms, here was the far more important type which not only proved the case, but extended the time-range and was so generalized that it could easily relate itself to the identical types leading into the early magnolias. Nor was the argument a vague one at all. The point or degree of staminate reduction remained uncertain. But the stamens were certainly small, the flowers at once proving the presence of a new and intensely interesting type, and indicating that the flowering gymnosperms were cosmopolitan throughout the Mesozoic.

Not content with these results, striking as they were, Nathorst at once set about examining the Rhaetic plants in hand still more critically by means of a technique of his own, applicable in the first instance to fossil plants partly carbonized. Depending on chemical treatment, with the use of collodion films, the "chemical method" of fossil plant study, as Nathorst in conversation preferred to call it, marked a momentous point in paleobotanical history, and has been since greatly varied and improved in the hands of other workers—leading at last to the nitro-cellulose method for the thin sectioning of the lignites as developed by Jeffrey. In reexamining the Swedish material and also fine collections of Williamsonians meanwhile made on the Yorkshire coast, by "chemical methods," Nathorst brought out in 1909 the finest demonstrations of cuticular and surface characters ever seen in fossil plants. These showed the histological features of the cone surface of the Skone cycadeoid to agree in the main with with those of *Cycadeoidea*, and also included beautiful preparations from the so-called "palisade ring" at the base of the "pyriform" axis of the cone (Pl. 4, Fig. 35,

P, Pr, the pyriform axis and "palisade ring.") In these new descriptions it became more than ever obvious that the slender stemmed Skone cycadeoids could not be retained in the genus *Williamsonia*, and the name *Wielandia* was suggested. This being preoccupied by a Euphorbia, was then rendered *Wielandiella*. It had been pointed out by Wieland in 1903 that retention in *Williamsonia* was unsound on the basis of either stems, leaves, or fruits.

The descriptions of the Skone plants, including the several species of *Wielandiella* as given by Nathorst, have the fine quality of being demonstrations of structure based on collections in the field, many years, in the making. They are much less arguments. As Nathorst remarked to me of the *Wielandiella* while going over his collections, "In a fossil, there is always missing that which one would most wish to see!" Too true.

However, Nathorst's redintegration of *Wielandiella* leaves the type the most significant "cycadophyte" known. The flowers must have had nearly the form shown in the two alternative restorations given on Plate 4. The general habitus of the plant was that of a magnolia, with respect to both branching and flowering; the foliage if taken as antecedent to the pinnately veined dicotyl leaf is of exactly a form befitting Rhaetic time; the small branched stems were in all probability of a primitive scalariform structure approaching *Trochodendron*. Quite the only feature which leaves *Wielandiella* within the gymnosperms at all is the cone. But Professor Mez is right when he says the cone might better be called generalized than specialized. It is at least most probable that the cone is derived from a uniserial type, the so-called "interseminal scales" being merely reduced from an early fertile condition. If so, the *Wielandiella*, and the quite different but also small flowered *Williamsoniella* of the Yorshire coast Lias, must have had many relatives from Permian times down, in which the seed cones consisted in simple series of uniformly fertile megasporophylls, these being of more distinctly carpellate form in the more and more primitive types. Pencil in hand, many of these hypothetic forms may surely be depicted, not a few of them as they once actually existed. A unit, or starting point in any such attempts at more vivid restoration is certainly afforded by the Winter stage of growth in Magnolia given in the appended Figure 1, which compare with the figures and provisional restorations of Plates III–V.

Moreover, going back in time, these amphistrobilar types would relate themselves not merely to other cycadeoids, but to the Cordaites, the conifers, especially as brought out in other paragraphs to *Cycadocarpidium*, and to that line which seems to end blindly in *Tumboa*. And too, no greater error can be made than to view the general course of change as one occurring mainly amongst megaphyllous forms. The exact opposite must be true; it is only specialized types that make up the bulk of the fossil series. Obviously the plastic forms of the past were not large and spiny; fossil occurrence being limited by that fact. The idea of great leaves, huge stems, and giant fertile fronds has no basis in fact. The Cordaites varied from types with leaves ten feet long to those with narrow grass-like blades. Giantism is merely a form of specialization found amongst all plants, and in all ages.

Hence, in coming forward in time from the days of the flowering gymnosperms, it must be believed that the more numerous and plastic types of cones and anthostrobili were as frail structures as are modern flowers. The carpel in the ancestor of the chestnut may have been leafy. The contrast between the flowers of *Drimys*, in some species about the size of those of the chickweed, and related flowers a foot across, has surely been paralleled many times in the geologic past. Putting aside large or woody types it is easy to imagine many small, fleshy forms of megasporophylls forming seed cones related to those of *Wielandiella* and *Williamsoniella* (=Microfloræ) and changing easily into the somewhat reduced form seen in *Anona glabra*, or again in a flower like that of *Asimina triloba*. The latter species has magnolia-like leaves; and stem structure not forbidding, no reason is discernible for excluding either genus from the Magnoliales. How early did strobili freely change or become reduced to the cyclic one-five carpellate form? That is the great question facing the morphologist. Perhaps the Dilleniales are so far as their actual *angiospermous* history goes, types *longer under way of change* than the magnolias.

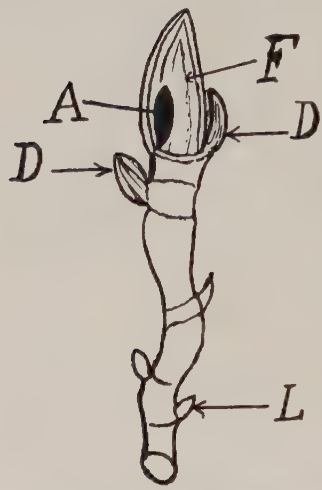

Fig. 1. *Magnolia Soulangeana.* Winter stage of flowering shoot with the type of false dichotomy characterizing *Wielandiella.* Compare with Plate III, and Plate IV, Figures 1–3. Recall that in *Williamsonia scotica* a bract inclosed vegetative bud is found at the seed cone base. L, latent buds; D, D, buds of the false dichotomy; A, apical bud (scale inclosed); F, the flower bud with stamens and seed cone well defined.

A Caution

How to deal with or view the plants of the past as indicative of lines of development is perhaps an art by itself, or a speculative philosophy that can only be discussed in its own limited terms. Taxonomists, nevertheless, have been freed from many of the hard conditions they faced in all earlier efforts to classify the angiosperms, by exactly the fossil discoveries of the past twenty-five years.

The diagram, Figure 2, serves to show from yet another angle how very discontinuous the fossil plant record must always remain. Evidently as plants reached complexity in geologic time the aggregate numbers of species increased in some approximately regular manner. It cannot of course be known whether there were in existence about the close of the Devonian 2,000 or 10,000 species of plants. The algæ and their descendants may then have shown an excessive variation, reminiscent of the first transmigrations from the sea. But, as diagrammatic methods indicate, there would be something like 50,000± species of plants existent in early Permian times, and over 100,000 at the beginning of Cretaceous time. Also, the species of plants may always be taken as in full course of evolution, so that their totality in all ages, and even the variation in families becomes to a rational degree a problem in mathematics.

Hence it is seen that the recovered fossil forms are as compared with genera and families in existence from age to age, represented by a narrow, ever varying area, as lined in to the left in Figure 2, and there greatly exaggerated. If it can be said, for instance, that 2,000 Jurassic plants are known in a fragmentary

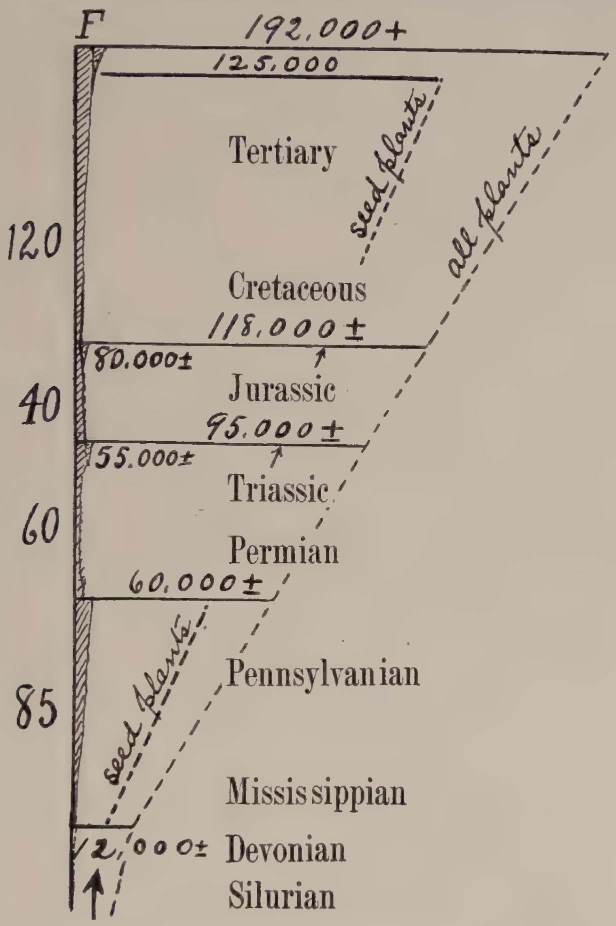

Fig. 2. Theoretic numbers of seed plant, and of all plant species from the Devonian to present time. The *argument* rests on the view that numbers of species must bear some average relation to complexity of structure, and that ferns, bryophytes, and thallophytes greatly increased in numbers of species as the forest canopy reached complexity of form.

The time scale is put at 85, 60, 40, and 120 million years for the Carboniferous, Permo-Triassic, Jurassic, and Cretaceous-Tertiary respectively. The hatched area F, to the left roughly indicates the manner in which the fossil record varies in bulk, but is exaggerated greatly with respect to the unknown (light area) forms.

manner, that merely means a record the more discontinuous because covering all of Jurassic time. The species occur in the smallest of florulites, widely separated, and throwing but the thinnest shadow over the course of change, certainly worldwide, leading toward the present day 264 families of angiosperms as recently listed by Hutchinson.

Polyphyly

Isolation of type is the first thing to consider in a fossil, chronology of structure, the second; followed by comparison with existent types, the third. Taking the magnolias, so magnificently represented in the Cretaceous rocks, in this manner, it appears a most practical thing to establish a great group, the Magnoliales. But the most remarkable member of the series as yet discernible must surely be *Trochodendron*, because its carpels may be, and its wood is primitive. Assumption correct, *Trochodendron* and the genus *Magnolia* are examples of parallelism rather than of late change. *Drimys* of the Winteraceæ is a third form widely separated by its wood, but related to *Trochodendron* from the viewpoint of leaf and flower, and in part the wood. It is quite possible that all seven families of the Magnoliales have been discrete since Jurassic time. The group is one of convenience, and in the present state of knowledge of it, defensible. But if the more strobiliform flowers initiated the series of changes into the present families, then the advanced floral types are chiefly many-seeded, and the accompanying wood by far the most primitive,—a contradiction not easily explained away by the idea of reversible evolution! No one can doubt for a moment that a tricarpellate *Talauma* cone is derived from some older and more typical *Magnolia* cone. But when it comes to viewing uni-, bi-, and actinicarpellate flowers generally as end-results of cone change, the starting point becomes less certain. Many cyclic or actinomorphic, and zygomorphic flowers may be old too.

Similarly with respect not merely to the entire woody phylum, but the herbaceous Ranaleans likewise. Floral complexity began in the gymnosperms, and is only seen in secondary stages in the angiosperms. One must recall that a flower like *Tumboa* is very old, that the Magnolias are not the only angiospermous group with an old, abundant, and cosmopolitan ancestry which has left a long and varied fossil record. And for that matter, what does absence of type in the fossil series signify anyhow so far as the evolution of the angiosperm families is concerned after Rhaetic time? Something, but not much.

The point of elemental change into actual angiospermous structure was reached when the older open strobili began to assume the cyclic carpellary emplacement. The carpellary leaves, large and small, then began to inclose their ovules few or many, meanwhile undergoing much change of form, with varied fusion. For the greater part, those were changes which might go on in all the early Mesozoic gymnosperm lines, and amongst types of carpels varied all the way from those of cycads to those of cycadeoids. But the cycadeoid "seed stem" itself was a carpellary type which either never reached the starting point, or else overshot the mark where elemental change into angiosperms occured.

From such a viewpoint the multiplicity of campanulate flowers becomes explicable as a result of changes which set in in the *pre-angiosperm* times, which paralleled rather than followed those ending in the cycadeoid companulate flowers. The inflorescent types also arose early in varied form. There must from every point of view be concealed within the angiosperms an extensive polyphyly. It is convenient and necessary to set the angiosperm house somewhat in

order, much as Hutchinson does and as Mez suggests. But it is certain that when the anatomy of flowers has been displayed, as is being done by Eames, and when all those facts are confronted with leaf-form, and with stem structure, made as searching and as elemental as Chodat would make the subject, it will be found that there are a score of lines quite as old as the Magnolias. That the demand for evidence of the origin of the angiosperms from within the angiosperms themselves must not be made too insistently was I think the substance of a very good remark Dr. Scott once made to me. And the very fact that Magnolias like *Talauma* seem to have shortened their cones but recently indicates that the Magnoliaceæ are just as much an arrested as a primitive dicotyledonous group. The origin of the flower in all its main types, and of the inflorescence in varied form in pre-angiosperm times is the virtual explanation of the appearance early in the Cretaceous of *Liquidambar*, *Populites*, *Platanus*, *Anisophyllum*, *Sassafras*, *Prunus*, *Fagus*, *Quercus*, *Salix*, *Juglans*, *Artocarpus*, *Laurus*, *Acer*, *Eucalyptus*, *Nymphæa*, *Diospyros*, *Fraxinus*, *Nerium*, *Viburnum*, *Cissites*, such monocots as *Alismacites*, and many other types traversing at least the greater part of the entire range of floral structure. Floral, foliar, and stem evolution was a process which must have gone on slowly in many lines, rather than rapidly and sporadically in a few. Sporadic change and specialization are merely opposite to the greater course of progressive change. It is not true that the paleobotanic record indicates either a late or a local or a rapid evolution of the angiosperm lines. It is true that there is glimpsed in the successive florulites a prevailing cast in vegetation from age to age. But to know what this means it is necessary to look behind the dissociated facts of the immediate foreground with exceeding care.

In the subjoined Figure 3, a further attempt is made to picture the often drawn "paleobotanic tree" but mainly with reference to origins. Such trees as are introduced in most present discussions of lines of descent do not look as arboreal

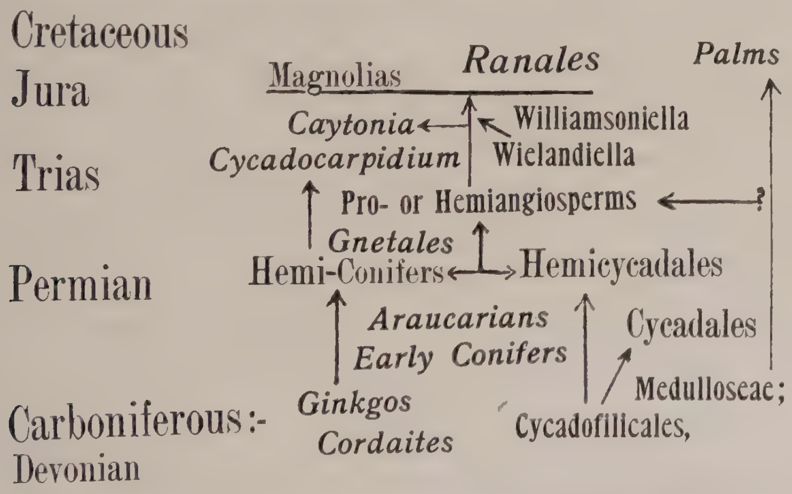

Fig. 3. Appearance of the Angiosperms in geologic time, with main lines of seed plants. A main purpose of this "tree" is to express the variant views of seed plant origin and descent.

as they did a few years ago. The older form of "tree" does not suffice for the fossil plants. A more general scheme is needed for the pre-Cretaceous plants especially, and yet one that allows detail, and is chronologic. What this scheme does is to juxtapose the ancient gymnosperm, and the modern angiosperm world, with due emphasis of the unknown in the era of transition,—in general, late Paleozoic to upper Mesozoic time. The necessity for broadening the angiosperm base and setting it further back in time is the contested point. Conifers followed closely by araucarians, with both appearing very early accords with the results of Florin. The Gnetales might be called hemiconifers. When they came to mimic the angiosperms is enigmatic,—perhaps in the Jura. Some ancient medullosan contact of the palms is suggested. The ginkgos must have followed the Cordaites closely, and antecedently to a later, extensive hypothetical group, the Hemiconifers, of which *Cycadocarpidium* might be an example, and *Caytonia* a derivative. The Angiosperms arise from the Hemicycad-Hemiconifer complex in the Trias by way of *Wielandiella* and *Caytonia*.

The Jurassic Plant Totality

In viewing Jurassic fossil vegetation with the object of marking out the course and extent of change precedent to the far better known Cretaceous plant alignment, numbers of species have a significance only second to structure. The plant totalities should be brought into the larger view where detail fails; else the negative, the unseen, an apparent absence of type, leads to a false visualization of the few types of the florulites, as compared with the many coexistent species, and thus vitiates every effort to bring to light approximate lines of descent as the prelude to a surer Taxonomy.

The fact has been emphasized that the Jurassic was a time of leaf and carpellary change in the earlier angiosperms, just as the Cretaceous was obviously the time of continued stem and floral change in the later, fully characteristic angiosperms; and it is also evident that the abundance of angiosperms in the Cretaceous rocks is due not merely to increasing complexity in the phylum, but to the greater thickness of the more and more recent plant beds. Hence it might long have seemed reasonable to suppose that the antecedent angiosperms were abundant in Jurassic time, even relatively dominant. But at this point the geologist seemed to tell the botanist that angiosperms originated locally and suddenly. Perhaps that vague pronouncement of the geologic texts was taken too literally; at least it led to fallacy, as may be shown from the rough estimate of Jurassic plant numbers, readily calculated as follows:—

As discussed in another topic, there were assumably in existence at the break of Jurassic time about 95,000± species of plants, at the very least, from a third to a half of these being seed plants; and by Cretaceous time perhaps 120,000 species all told. Allow these numbers as an approach to reality, letting a equal the first, l the second. Note that some forms change much, others not visibly at all, but that an average of end-on-end specific changes, for all of Jurassic time might be fairly taken as $10 = n$. Note also that in a progression, generally expressed, there may be disregarded the following: variation in num-

bers of species due to excessive specialization, *sp*; great increase or decrease of species due to altitude of the land masses, *ay*; high or low numbers of species due to bathmism, *bz*; other theoretic and variable factors, $=\acute{n}$. Then, S being the Jurassic plant species in totality which it is desired to find, this formula becomes available:

$$S=\frac{(l+a)n}{2}+sp\pm ay\pm bz\cdots\acute{n}.$$

Substituting the values above indicated:

$$S=\frac{(120{,}000+95{,}000)10}{2}=\pm 1{,}075{,}000.$$

That is, the well marked plant species of all Jurassic time, as inclusively taken in the many families and genera leading into the initial Cretaceous alignment, roughly approximate one million. It would seem impossible that the number could have been as high as 5,000,000, or as low as 200,000. Of the possible million species, few over 1,000 have been seen as fossils definitively assignable to their phyla.[3]

That is, perhaps a tenth of one per cent of types existent in that stretch of geologic time is recorded by dissociated leaves, stems, and rarely the flowers or fruits. The latter occur only in the widely separated florulites; consecutive types are seldom if ever seen, unless ages apart; instances of the fossilization of all parts of any of the seed plants are only seen at the vanishing point of the record. That is why the investigator on passing beyond the familiar stem and leaf types of present forests seems left without a footing. That is why the stems and flowers of the tens of thousands of species in the antecedent angiospermous floras are not seen; and that is why it is worth while to make the severest analysis of such adumbrant forms as are found.

Reversible Evolution

Change as observed in fossils has usually led to belief in irreversible descent. Botanists, however, may question views so largely based on the invertebrate and vertebrate sequence. Plants are, despite a certain multiplicity of form, so generalized in their environmental adaptations that the recessive tendency easily becomes marked. Thus in the case of the simpler dicotyl stems it may be that the view that scalariform wood is very generally primitive, is too sharply limited. Some botanists believe an opposed course of pit fusion the more significant. Both changes can occur, and it is probable that relative amounts of scalariform and pit wood sometimes fluctuate back and forth.

Hallier, who perhaps more than any one else has correctly stressed the pri-

[3] This would be the general result if the seed plants alone were taken, using the data of Figure 2. For then the pteridophytic elements would be subtracted from the florulites; and it is found that in older Mesozoic floras about 40 per cent of the species are ferns or fern allies. It so chances that the species below the seed plants number close to 40 per cent of the described existent plants.

mitive carpel as the unit in the evolution of the angiospermous flower, has called attention to the leafy flowers of *Aquilegia canadensis* which occur variably. His view that the carpellary expansions are reminiscent of the early types, going all the way back to the carpels of *Cycas*, seems correct. But the reversion itself is structurally dicotyledonous. It is generally the modern net venation that is carried back onto the flower; or in a cycad cone it may be the fronds even, as in Figure 4.

Fig. 4. Extremes of carpellary variation in cycads. 1, foliar recessiveness in the cone of *Encephalartos villosus;* 2, carpel variation in *Zamia floridana;* 3, bifurcate seed stems in *Zamiostrobus stenorhachis* of the Rhätic; 4, *Cycadospadix Hennoquei* of the Lias, a variant from *Cycas*.

The number of ovules to the carpel must have played a great part in floral development, and must still vary back and forth with some very marked effects on outer form. Particularly if the strobilar origin of the flower were held to very exactly, the fact that the megasporophylls of strobili are so universally few seeded might mean frequent late reversion amongst angiosperms to many seeded forms. That is, with reduction of the strobilus to the cyclic or floral form, it would seem that carpels frequently regained the many-seeded condition. But there stands opposed the fact that the magnolias, while undoubtedly retaining ancient carpellary features, may have held to the strobilar organization too fixedly, too long to give origin to many of the commoner types of flowers, especially those with united many seeded carpels. However this may be, the

point is raised that in floral structures there may be an approximate return to an earlier stage which then becomes the basis of new change. Also, members fused into a campanula might again become discrete. The flowers of the maple might again become tricarpellary; the stipulary sac of the hop hornbeam and of the hazel might divide. Many of the difform leaves and flowers might again take on regularity. An axillary inflorescence may again be reduced to the primitive single flower as Parkin notes ('23) in *Anonis Vicia*. The basal flower of the original raceme is the one retained in the apical position after a completed cycle of numerical change.

An absolute repetition of a course of evolutionary change can never occur. But reverse change in number and to a certain extent in form in floral organs has been of profound significance in the evolution of the angiosperms. Modes of origin of present day types are many. However convenient it may be to place the Magnoliales and the Ranales at the base of the angiospermous series the monocots form a third very old line, and it is likely that all three together only account for a fraction of the flowering plants.

The Older Floral Morphology

It was but yesterday that astonishing chapters were written into the history of the early land plants by Kidston and Lang, and by Kraeusel. Chodat shows how utterly important and elemental the Rhynias are in ideas of stem origin. Chance favoring likewise, when perhaps 50 Triassic angiospermous fruit types have been found they must, as it were, throw a shadow along the greater course of events. The outlines of angiosperm evolution must become safely inferable. Even now it seems that floral structures rather than leaf or stem, must furnish the more critical evidence for the main course of change leading to the modern types; but until of late there has been little assurance as to the order of events, the manner in which the angiospermous emplacement of organs has arisen. It was therefore startling to find in the cycadeoids that disks are so old, to see there flowers which are such in all respects as compared with those of angiosperms, to find that stamens can be so frond-like, to see in Cycas that the entire crown is strobilar and thus to glimpse courses of reduction as well as phenomena of gigantism. It was helpful to see evidence for the nature of transition to the axis of limited growth, the short shoot, the cone, and the inflorescence; to note that relative size of organs as tied up with branching is so much of an evolutionary factor. It proves illuminating to learn from the careful examination of the fossil record by Florin that both pines and Araucarias are really old—doubtless Paleozoic in origin. And still speaking of forms without the angiosperms, one ancient form we could wish to know more about than any other is the curious microspore-bearing disk from the Pennsylvanian called *Codonotheca*. It is, however, from within the angiosperms themselves that the new evidence is wished.

Geographic Limits to Fossil Discovery

The future holds every hope of discovery of the primitive angiosperms; perhaps the man destined to find the lignitized stems of the *Wielandiella* or of the

early magnolias is in this audience. Certainly such a discovery would hold very great scientific value; although it is so evident, that a very closely connected record of the plant life of the past cannot be expected. Bailey and Sinnott have pointed out in what is held a sound manner, the probability that the elevated regions of the earth have long been regions of active plant change; which is neither more nor less than a corollary to ideas of northern origins, of or origin within the polar areas, often advanced. Naturally the times of more extensive arctic lands, and of greater continental and plateau areas, with the more stressed climates, tend, unfortunately for the paleobotanist, to leave the lesser fossil record. The plastic plants of the high latitudes and the high altitudes are apt to fail in the fossil lists; the Ericaceæ may be an example. Whereas, the more average subtropic vegetation with many specialized types leaves the more conspicuous fossil record of those times when the continents are low, and there are large bodies of fresh water, and when climates are in part warm. This general fact has often led to imperfect views of ancient climates. But it is only a general fact at most, and as already emphasized the fossils will yet throw a defining shadow over the course of Permo-Carboniferous and early Mesozoic plant life. The tendency of arid or desert regions to leave a great petrified record is a partly compensating fact.

Rhaetic Dicotyls

Progress in the investigation of the older angiosperms depends on special horizons and localities far more than in the case of either the vertebrates or the invertebrates. Fossil plant preservation is with the exception of some of the petrified types so generally precarious that it will in the end be necessary to search the world over for the localities favoring special types of plant fossilization. Every advance in the methods of fossil plant study, every structural feature so far determined points to the truth of this dictum, which can be no better illustrated than by briefly describing several new fruit species from the plant beds which outcrop at the Minas de Petroleo, southwest of Mendoza, Argentina, and at Cacheuta. These beds, about 160 meters thick, are of a three-fold interest. Especially the lower portions are finely laminated, perhaps seasonally so, and contain insects which must prove numerous, as well as many plants in delicate detail. The plants have not been collected or studied with anywhere near adequate care, though in the main suggesting agreement with other series of Rhaetic plants. The insects are certainly also present in variety. The first of them were discovered and described by myself, with however, the corrections and help of both Cockerell and Tillyard, two foremost authorities on the fossil insects. Exemplifying the vicissitudes of fossil preservation and discovery, so needful for the fossil botanist to ever hold in mind, these fossil insect finds of mine were the first ever made in the entire continent of South America; and they ('26) also show no features out of accord with Rhaetic insect life. These fossil series mark the Argentine Rhaetic as one of the most important fossil localities in the world, one even equaling in interest the Rhaetic of southern Sweden, the Lias of

Mexico, and the region of the Gondwanas of India. It is the Rhaetic which contains the oldest, the remotest evidence of angiosperm floral features yet found.

Amongst the handsome plants secured at the Minas de Petroleo there are numerous alate seeds or rather fruits (Fig. 5) which I for a time thought might belong to some ancient conifer. But it now seems that these fruits may be dicotyledonous because of a certain resemblance in size, form, and feature to the fruits of *Fraxinus*, the ash. No such seeds have ever been reported or figured from any South American horizon. There is in the lamina, as shown in the figures, a certain resemblance to *Cycadocarpidium*; other comparable fossils are several fruits more or less closely associated with leaves of *Fraxinus*, described

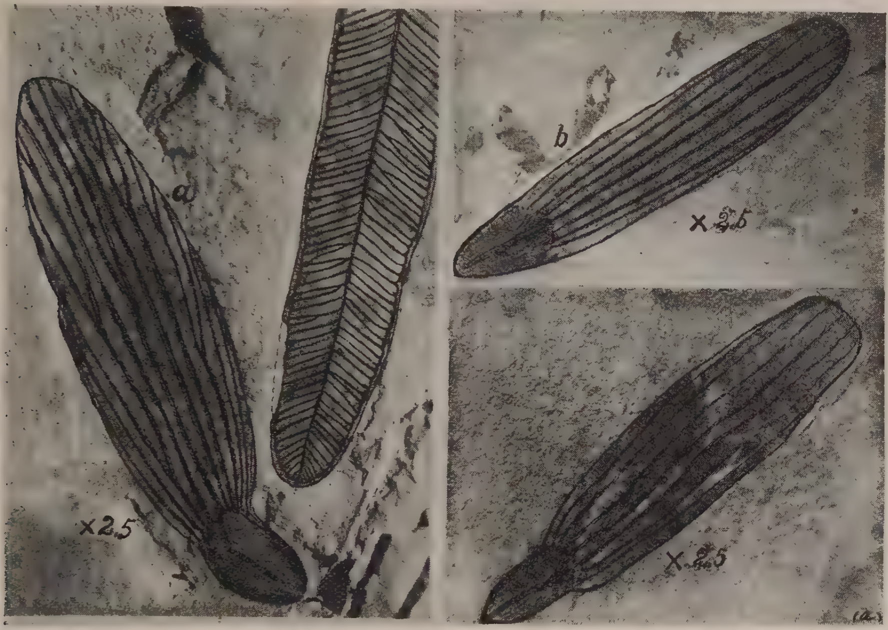

Fig. 5. New seed or fruit types from the Rhaetic strata of the Minas de Petroleo, southwest of Mendoza, Argentina. ×2.5.

a. Fraxinopsis major associated with foliage showing *Stangeria*-like venation, but probably dicotyledonous. [Generic name of foliage left in abeyance,—not *Taeniopteris*].

b. Fraxinopsis minor, two of a large number of specimens.

by Heer, Saporta, and Unger. *Fraxinus predicta*, *F. juglandina*, and *F. gracilis* all consist in both leaf and fruit, and are Pliocene. *F. macrophylla* of the Greenland Tertiary is older; while the leaf type runs back to *F. praecox*, of the Patoot, and *F. denticulata* of the still older Atané beds.

These fruits occur in the lower third of the outcrop at the Minas de Petroleo, in the closest association with the fossil insects. There are two types, a smaller abundant form of which about ten examples were found, and a distinct larger pattern only found several times. In any case, as shown in Figure 5, *a* and *b*, these are unique, determinative species which because of their symmetric and

highly developed venation may be dicotyledonous. Conifer seeds are quite generally asymmetric, and without such clear venation. As along with primitiveness a certain resemblance to the ash is suggested, the convenient, conventional names ***Fraxinopsis Major***, and ***Fraxinopsis Minor*** are given the larger and lesser type respectively.

In *Fraxinopsis minor* the leafy expansion is in nearly every instance traversed by seven nearly parallel veins running the full length of the ala. The midvein may be slightly the strongest. There is a slight sculpturing of the seed end, as if there might be two cotyledons of rather large size present. Under the binocular it is noted that the matrix has favored preservation of surface features which very well agree with those of the ash.

No stigmatic surfaces can be detected at the end of the midvein; but such could come near the seed end where they would be difficult to see. In the ash the position of the stigma is merely indicated by an indurated point carried forward by the growth of the ala. The fossil being a cast and imprint, the observable features are merely those figured.

The *Fraxinopsis major* is a very fine fossil, more robust than, and as symmetric as the *F. minor*, the oblique set of the ala being merely due to some maceration. In this species the midvein forks once. Then come two single veins running the full length of the ala. Next the single vein comes a triply forked vein on the one side of the ala, and on the other, two twice forked veins, with lesser border veins. That is, while in the lesser seed the venation is simply parallel, in this larger form there is marked dichotomy.

A third species of these fruits evidently occurs in the Rhaetic outcrop at Cacheuta, some miles away from the Minas de Petroleo. The original I have not seen; but in a group of Cacheuta specimens figured in an Atlas[4] of Argentine fossil plants a blade with slightly forked venation is shown which has features intermediate to the foregiong fruit species. Though the drawing alone is too uncertain to permit the naming of this species, there is the same association with fossil insects and other plants.

These notes on the Rhaetic fossils of the Minas de Petroleo and Cacheuta only serve to emphasize the fact that the investigation of the remarkable fauna and flora there found should begin in the field at the localities themselves, and there alone. And the fact cannot be too strongly insisted upon that such field work must be done by the investigator himself. Only with the advantage of collection in the field followed by description based on the field notes can the fuller quota of evidence afforded by these or any other more important localities be brought to light. The isolated evidence from lesser localities must often be examined by some one else than the original collector. But in all greater localities, the investigator must collect his own material. Else many, even the most crucial facts must remain obscure, and not merely paleobotanic, but important geologic data may fail of recognition entirely. In the present instance, I should have little conception of the importance of the Argentine Rhaetic from any mention found anywhere in the literature.

[4] Actas de la Acad. Nacional de Ciencias de Cordoba **7**: 129–153. 1921.

Collateral Theory

While giving the foregoing description of new Rhaetic fruits the thought has perhaps been near that thus might an over-speculative treatment of a subject, at best recondite, be saved from a speedy scientific oblivion. Nevertheless, it may be that a new viewpoint is here reached; for, as noted, the blade of the *Fraxinopsis* fruit or carpel bears a certain resemblance to that of the generalized gymnosperm of Rhaetic times known as *Cycadocarpidium*. This fossil as restored by Nathorst (Fig. 6) was one of the lower Mesozoic gymnosperms with lax cones, and is usually regarded as more or less of a composite between cycads and conifers.

Fig. 6. *Cycadocarpidium Erdmanni* Nathorst, from the Rhätic of Skone. 1, carpel bearing two seeds, natural size; 2, 3, 4, other carpels enlarged; A, restoration of strobilus, or anthostrobilus. [One of the lesser Cycadocarpidiums.]

Now if the interpretation of the "Fraxinopsis" is correct the seed is enclosed, the entire structure a fruit, and the idea of a pair of scars left by seeds borne basally to the ala is inadmissible. None the less, fruits and carpels may not have been far apart in the primitive floral, and lax cone types of Triassic forests. Exactly too, in *Cycadocarpidium* there occur in some of the rather varied species leafy bodies on which the seeds are seated at the base of the main carpellary blade, so that any infolding of these leaflets about the seed would at once result in an inclosed, sub-angiospermous condition. Moreover, Dr. Florin has called my attention to the fact that the *Cycadocarpidium* carpels sometimes bear three or four seeds on each side of the axial line, whence with reduction of the larger blade, and infolding of the leaflets about the seed groups, something much like the *Caytonia* structure (Fig. 7) would be reached. Though according to the view here suggested, this if identically the fact would not mean that after such a change *Caytonia* had still remained a gymnosperm.

Permo-Triassic time, the age of lax cones and free carpels, surely limits the period during which the main angiospermous groups appeared. That is, so far as evidence and theory go, the critical stem, leaf, and floral changes forming the

basis of angiosperm evolution had been accomplished by the beginning of Jurassic time. The Jurassic was especially the period when the archaic and transition angiosperms were gradually replaced by the forms quite like those of today. Cretaceous and Tertiary time as already remarked saw the rise and distribution of the present families of flowering plants, and was thus a time of new genera and species. More than ever, it is the carpel which is of supreme interest in any discussion of angiosperm antiquity, and it is quite evident that in searching out antecedent forms botanists must go back to the lax cone and free carpel—hence to the time when there was in reality far less distinction between Cordaites, Ginkgos, Conifers, Cycads, and Cycadeoids than is common to our every-day picturing. The question whether the angiosperms are descended from any one of these groups is academic. The angiosperms are somewhere descended from all of them!

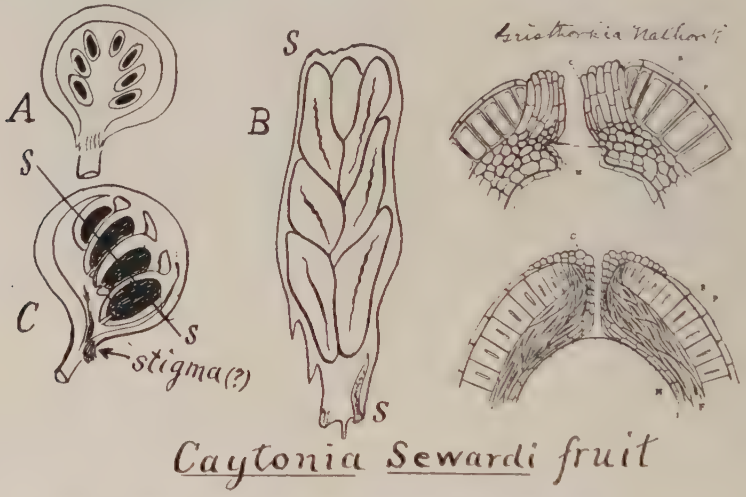

Fig. 7. *Caytonia Sewardi* fruit (left), and *Gristhorpia Nathorsti* seeds in longitudinal section (right), the new supposedly angiospermous types from the middle Jurassic of the Yorkshire coast. After Thomas. [Closely associated foliage, *Sagenopteris*.] Fruits enlarged 3 times, seeds 50 times.

A, position of seeds in fruit; C, diagrammatic view showing position of seeds in fruit with strands of cutinized cells leading in from supposed stigmatic point, with line *ss* indicating position of transverse section, B; B, camera lucida drawing of transverse section through fruit showing position of seeds.

Nor is the kind of change that went on during the age of the free carpel so very much hidden. It was bound up with transition from the spiral to the whorled, and finally the cyclic insertion. The end to attain was the production of the enclosed seed in the flower, often reduced, borne in incomparably greater numbers than were the earlier cones, and chiefly characterized by the varied types of carpellary fusion. The staminate changes were simple, reduction, in so far as that ever was concerned in the evolution of the angiospermous stamen, having been already accomplished or present in Cordaite days. The amphisporangiate habit, moreover, easily arose, and is very old. It is enough to cite this

habit in types so diverse as the cycadeoids and *Tumboa*, with also sporadic reversion to amphisporangiate strobili in conifers (Fig. 8). That amphisporangiate fructification was vastly more prevalent in higher forest types from the Permian down than the fossil record discloses, must be believed.

In the older gymnosperm times seeds were borne nearly free on stems either simple or reduced as in Cordaites, ginkgo, and apparently also the cycadeoids, or else on leaves or fronds. But certainly the very fixed impression is gained that carpellary organs were more leafy and varied in pre-Jurassic times than later, and that during those times of the carpellary leaf when gymnospermy had reached its peak two great courses of change become recognizable—the one, a course of specialization into woody cones, the other a plastic series of changes into the many types of cones and whorls with the forms of carpel fusion seen in

Fig. 8. Proliferation and amphisporangy in conifers. Left to right: Proliferation in *Larix*; normal ovulate, shortened bract-inclosed ovulate, normal staminate, and amphisporangiate cones from a small *Picea Alcockiana* tree. The shortened ovulate cone is a part of the assumed recessiveness in the return to an amphisporangiate condition; although a normally amphisporangiate conifer or *hemiconifer* remains to be discovered.

the modern flowering plants. The coördinated stem and foliar change is readily understood, is in fact for the greater part already attested by fossils if fairly compared with existent types.

Much of the change toward modern families no doubt went on with an excessive slowness, so that in the course of time even generic distinctions arose without the intervening species usually assumed present. That sudden reductions, as well as sudden multiplications of parts, that is saltations, also occurred and became fixed, may be suspected. Furthermore, while the fertile organs of plants must always have remained such through all the vicissitudes of descent, and while it is well recognized that regrouping of organs eventually

occurs, little attention has been given to a certain recurrence of foliar features in cones and flowers. The phytologic advance has always been to some extent reflected in fruiting organs. Thus in part may be explained the origin of seed coats, floral envelopes, leafy carpels, and the leafy expansions in such fruits as those of the maple, ash and hazel. As a consequence it is not always apparent whether a given feature is primitive or the result of this reflex or secondary growth, often ending in a kind of giantism. Thus for instance the most difficult of all flowers to explain are those of *Cycadeoidea* with stamens large and complex enough to be the small fertile fronds of some Marattia-like fern, and yet with macrosporophylls as reduced as those of Cordaite, or early Ginkgoids.

Considering, however, the fact that fusions so readily take place between fertile segments, or between leafy or stipular outgrowths, it is probable that entirely too much has been made of the distinction between the naked and the closed seed. Any infolding of carpellary margins, or fusions of such, might easily be accompanied by micropylar elongation and indeed the direct evolution of the style.

In thus considering the carpel and carpellary emplacement as the great features in the evolution of the flower a more facile viewpoint is reached. The older seed-bearing plants are brought into greater unity, and the development of the gymnospermous seed itself becomes a step toward later floral evolution. While, as Thiselton-Dyer expresses the point, ". . . . an ovule is a sporangial structure, and it is not easy to see anything in a pinna which is in any way comparable to it. Morphological conceptions must not enslave us, and I see no reason why sporangial structures, like buds, may not appear anywhere." The most primitive of all flowers, that of *Tumboa*, should also be brought into this view; and what may be strongly suspected is that the organs leading towards flowers, and the flowers that have come down through the ages were generally small and reduced. In all considerations of the origin of floral structure as tied up with old gymnosperm lines, the problem of megaphylly versus microphylly is ever present. It is certain since the discovery of the macrophyllous seed ferns and the macrophyllous stamens of *Cycadeoidea*, that megaphylly was widespread amongst ancient seed-bearing plants. But the view is advanced here that this problem only has to do with variation within the several groups, that every one of the great gymnosperm groups includes types that long remained megaphyllous, and that all more decided forms of megaphylly indicate a specialization beyond the actual lines of progressive change, that is lines concerned in the evolution of the higher seed plants. There is always present the question of reduction, progressive advance, and the reflection of the vegetative changes in fertile organs. Sahni, remarks at the close of an elementally interesting study of the monotypic conifer of New Caledonia, *Acmopyle Pancheri*, that,—"No definite conclusion is expressed on whether the Conifers ultimately arose from microphyllous or megaphyllous (fern-like) ancestors; for while it is believed that they arose from Cordaitalean stock, the origin of the Cordaitales themselves from a megaphyllous (fern-like) stock is still considered to be "not proven on the existing evidence." This is only as it should be. Both facts, indeed all three facts,

megaphyllous and microphyllous ferns precedent to the Cordaites, and a certain degree of Cordaite contact in the precedent conifers, must each and all be true. Plant evolution is made to look unduly complicated when the fact is too much neglected that fossil types are few, lines old and varied, with progressive evolution dependent on the unspecialized.

Botanists and paleobotanists alike have drawn on the taxonomic evidence in too restricted a manner. They long accepted as generalizations approaching an etermal sublimity those dicta of geologists so definitely clogging the way leading toward an understanding of the plants of the past. Those fetidly tropic climates of old were not universal. The Carboniferous forests were of immensely varied character, the inland and upland types never having been seen. Gay floral colors must go back to the Permian. The "age of cycads" was always a misnomer and a myth. Lower Cretaceous times absolutely did not witness the rise of the flowering plants. Nor do plants evolve like horses, or migrate like mastodons and elephants. It is the peculiar quality of the long plant record, outlasting the deposition of some 225,000 feet of sedimentary rocks, that it is so often histologic, and that it is firstly to be considered in terms of itself. At no time since DeCandolle began to stress the morphologic taxonomy have the angiosperms looked so uniform, so homogeneous, so certainly derived from a fixed position within the plant life of the past, or so distinctly the resultant of the changing geologic environment as now. "Plants" as Bessey said, "are related up and down the genetic lines, and the system to be quite natural must recognize these lines."

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Explanation of Plates

PLATE I.

Petal, stamen, carpel, and bract structure in cycadeoids. Compare Plate II.

Fig. I. Transverse section of campanula segment in *Cycadeoidea dacotensis* from above insertion, showing single outer row of xylem strands of the fused members of the corolla (C, C′), and the bundle patterns of the stamens (S, S′). In the latter the frond fusion is at (*f*, *f*′), and the interior frond bundles are especially to be noted at *v*. The pattern of the stamen bundles does not vary greatly from that of the leaf bases in certain species. See Figures II and III. × 10.

Fig. II. One of the smaller of the xylem strands of the outer ring, see preceding figure (Fig. I).

Fig. III. One of the larger strands of the stamen pattern, with protoxylem distinct. This and preceding figure are camera lucida drawings made at 625 diameters. Xylem only preserved. [Higher in the campanula the inner staminate bundles tend to drop out and leave a somewhat two-rowed condition just before the corolla and stamen series become discrete.] Figures II and III are × 250 ±.

a. Transverse section of the seed stem of *Bennettites Moriérei* cut just below a seed. The sclerotized layer of cells E does not belong to the seed stem, but is the outer layer of the closely adpressed inter-seminal scales, four to six in number. × 60.

b. Bract structure with one of the bract bundles. × 65.

PLATE II

Floral Structure in *Cycadeoidea*.

Above.—*Cycadeoidea ingens* flower as restored in glass at the Field Columbian Museum, on the basis of the Yale Specimens. Somewhat reduced in size.

Below.—Transverse section through mid-region of central seed cone of another species, *Cycadeoidea dacotensis*, enlarged. Here the disk has been shed, the first stage in the course of fossilization beginning after that last event in the life of the plant.

PLATE III

Wielandiella, *Williamsoniella*, and *Cycadeoidea*. Habitus, floral features, and seed cone structure.

Fig. 1. *Wielandiella angustifolia* from the Rhätic of Skone, as restored by Nathorst. There is some doubt about the cone axis extending back to the third bifurcation, or thus persisting to the third, instead of second year's growth as in *Magnolia*.

Fig. 2. *Williamsoniella coronata* from the Inferior Oölite of the Yorkshire coast, as restored by Thomas ('25). At F, the stalked, apparently naked flower, several times enlarged; at T, the *Taeniopteris vittata* foliage. The association of this foliage with the forked-flower-bearing stems is as close as that of the *Anomozamites* fronds and the *Wielandiella* flowers, but is less determinative. In any case the *Williamsoniella* leaves were approximately of the character shown.

Fig. 3. Longitudinal section of seed cone of *Cycadeoidea*. Only remnants of disk (*S*) remain. It is believed that the seed cones of both *Williamsoniella*, and *Wielandiella* agree in outward form with this type of cone, but that the embryogeny was advanced toward that of dicotyls.

PLATE IV

Floral structure in *Wielandiella* and *Magnolia*, with two trial restorations of flower of former.

Fig. I. First trial restoration of the flower of *Wielandiella angustifolia*. About natural size, and based on one of the stems with buds, or fruit scars. R, receptacle as seen in fossil; St, stamen of large size and primitive form; B, upper bud of a false dichotomy supposedly inclosed by the bracts, much as in the Winter flower buds of *Magnolia*, and as seen in the Cycadeoid, *Williamsonia scotica*.

Fig. II. Second trial restoration of *Wielandiella angustifolia* flower. At F, old flower bud or peduncle base, with small scars possibly left by stamens. Leaf buds B, of the false dichotomy not inclosed by lower bracts. Stamens St, in position where pollen was found, and reduced to about the *Magnolia* stage, in several whorls not forming a collar. In latter case the scars of old peduncle base F, are left by the perianth. Not believed so nearly correct as I. Compare both I and II with the four drawings from the actual fossil, Figure III.

Fig. III. Four drawings of *Wielandiella angustifolia*, forked stems with peduncle bases and fruits partly fossilized. These figures are from Nathorst ('02), and the numbers 2, 4, 18, 20, correspond to the figures of Nathorst's Plate 2. Features are merely sketched freehand to show interpretation now suggested, as follows: e, floral envelope; st, stamen scars; Pr, the so-called "Palisade ring" on which pollen of cycadeoid and *Magnolia* type was found, but likely to prove portions of persisting sterile basal organs of seed cone. P, pear-shaped prolongation, structureless, but doubtless exactly outlining the receptacular cushion. Compare with IV.

Fig. IV. *Michelia montana*. Perianth scars, P; Stamens St, on left, and abbreviated region of stamen scars at base of gynophore on right. Seed cone reduced to tricarpellate condition. After Blume, Florae Javae. See next plate.

PLATE V

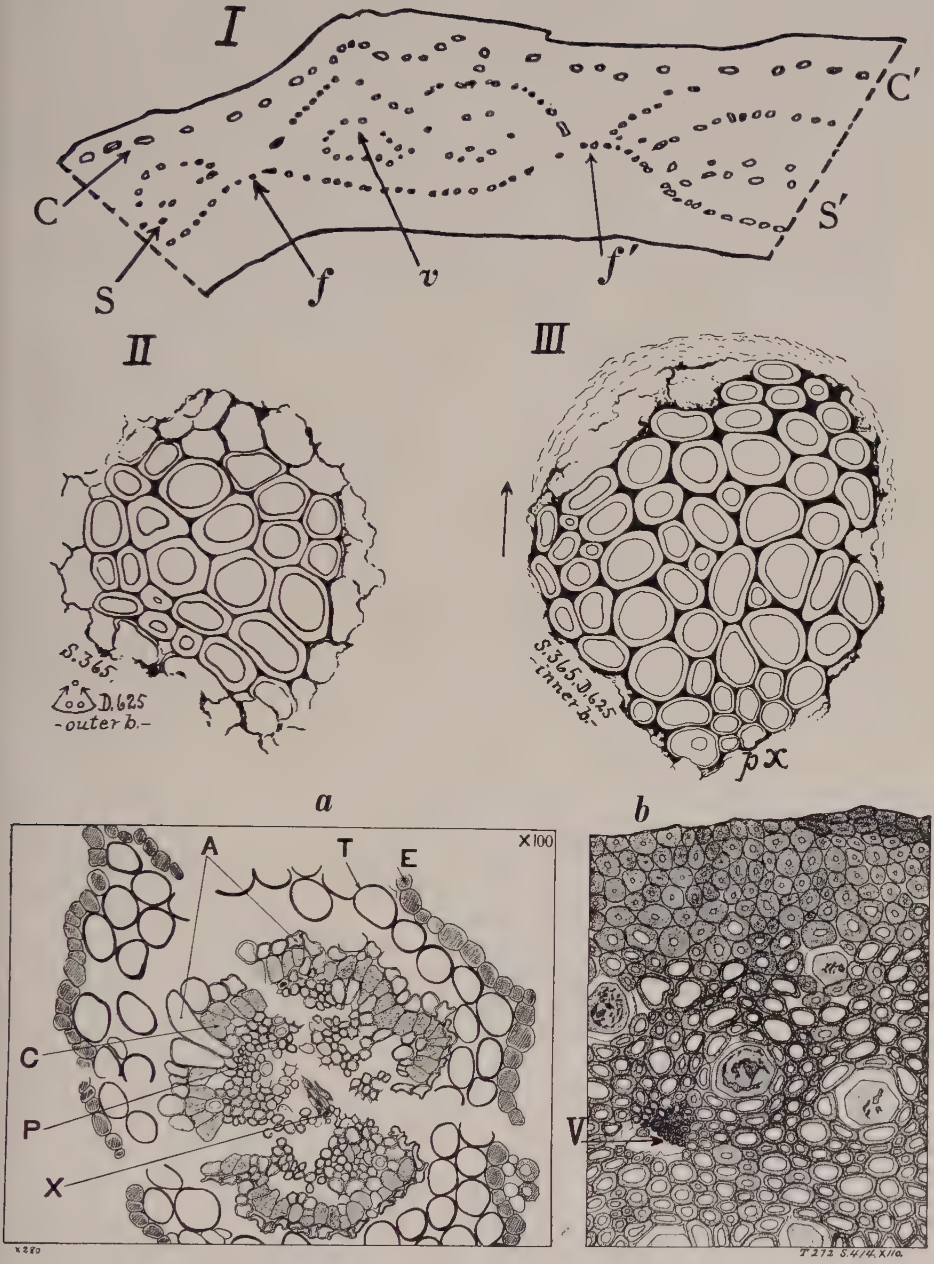
Floral structures and apical growth in the magnolias compared with cycadeoids.

Fig. A. *Michelia Cathcarti*, showing suppressed perianth scars (P), followed by prominent stamen scars, and slender cone on short gynophore; (a) pollen grains more like those of cycadeoids than in most angiosperms.

Fig. B. *Michelia pubinerva*, two drawings showing prominent perianth scars, stamens (St, St), and seed cone (sc).

Fig. C. *Magnolia tripetala* showing leaf and peduncle scars (E), with departure from pronounced branch dichotomy.

Figs. D1, D2. *Magnolia Campbelli* (D1, flower less perianth; D2, seed cone) showing heavy perianth and stamen scars. These figures are about natural size, and show the great mass of stamens, the large perianth scars, P, and stamen scars S. Enlarged view of single stamen, St, and of denticulate termination of young carpel, Ct. In D2 only lower portion of seed cone is shown. [Both the flowers and mature cones exceed in size those of *Wielandiella* and *Williamsoniella* by far.]



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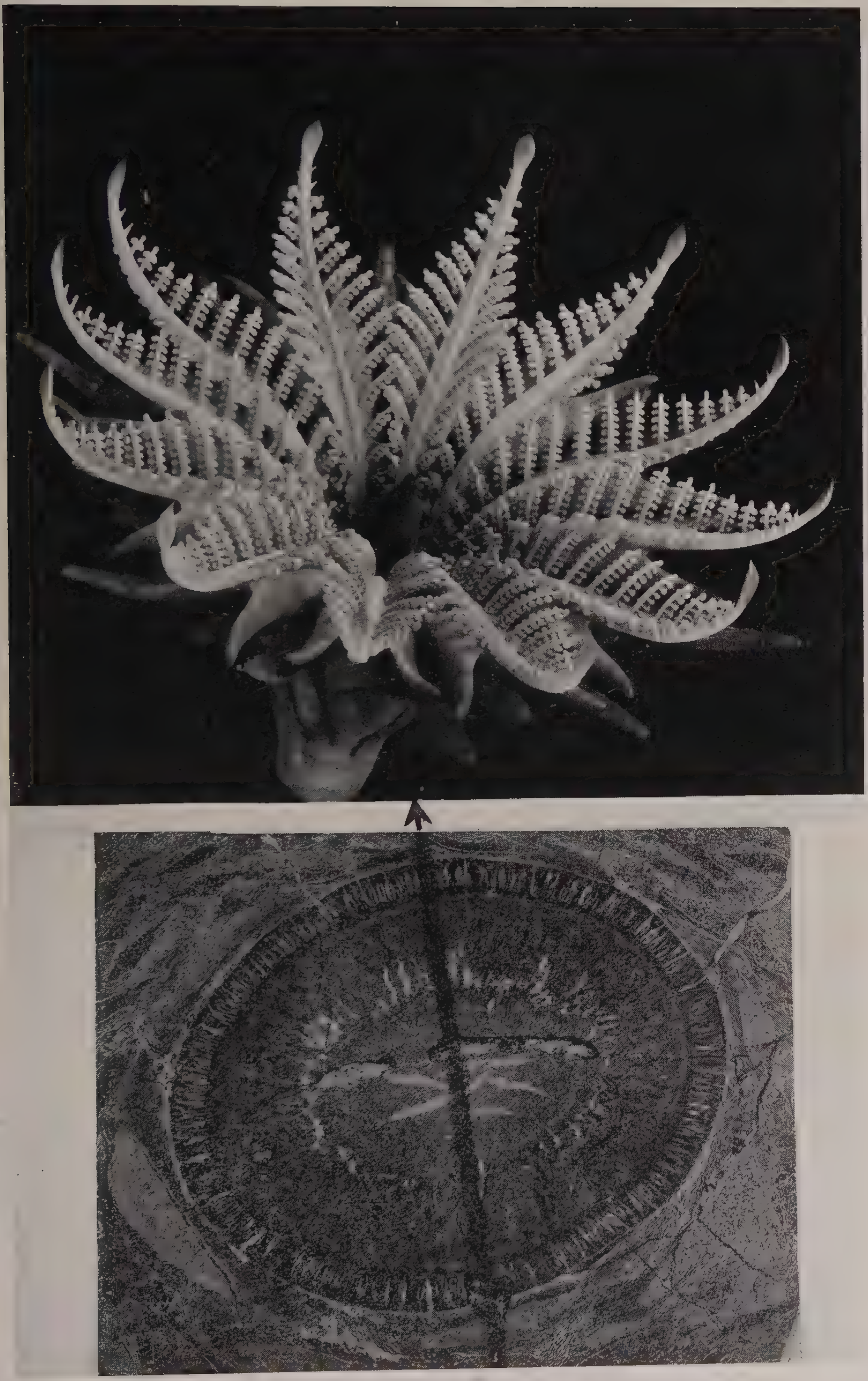

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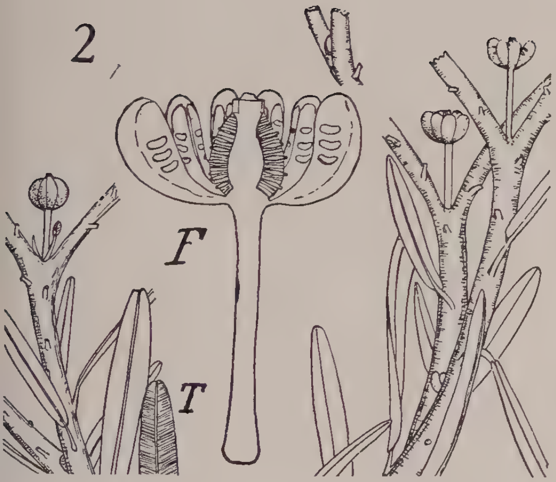

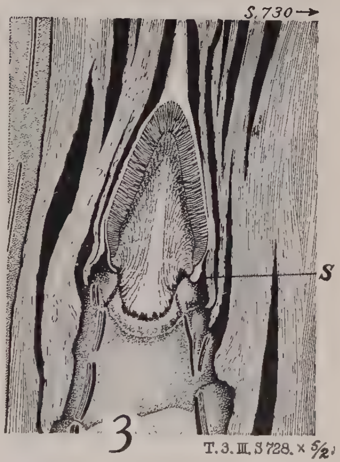

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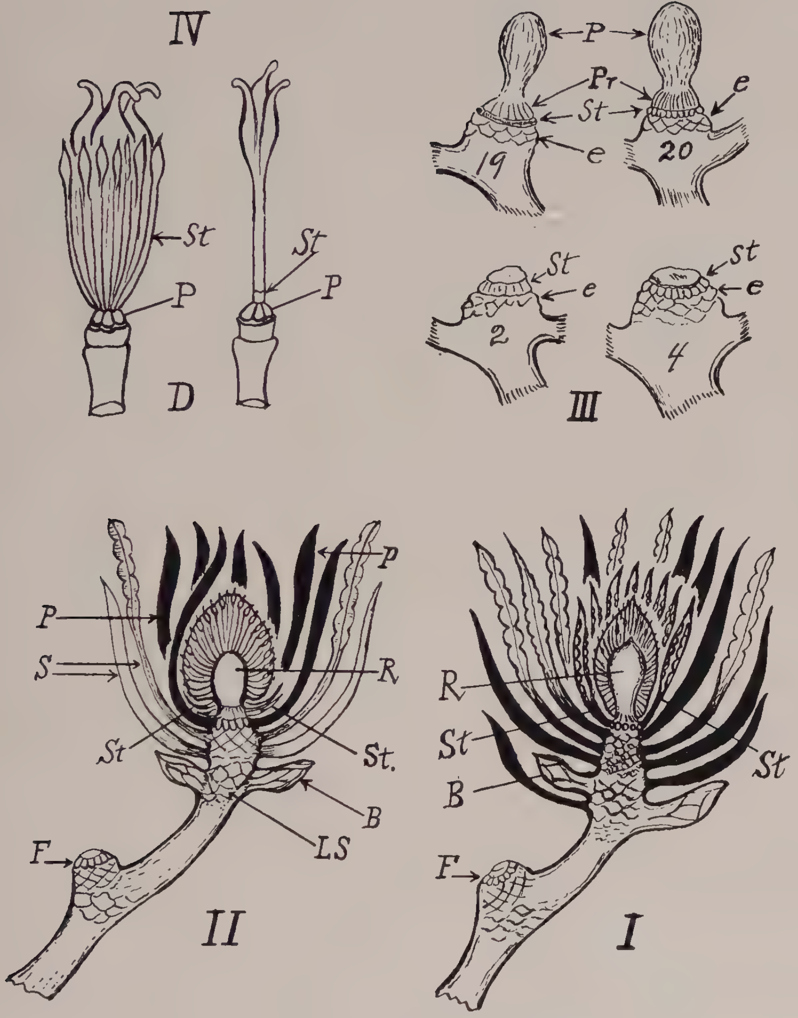

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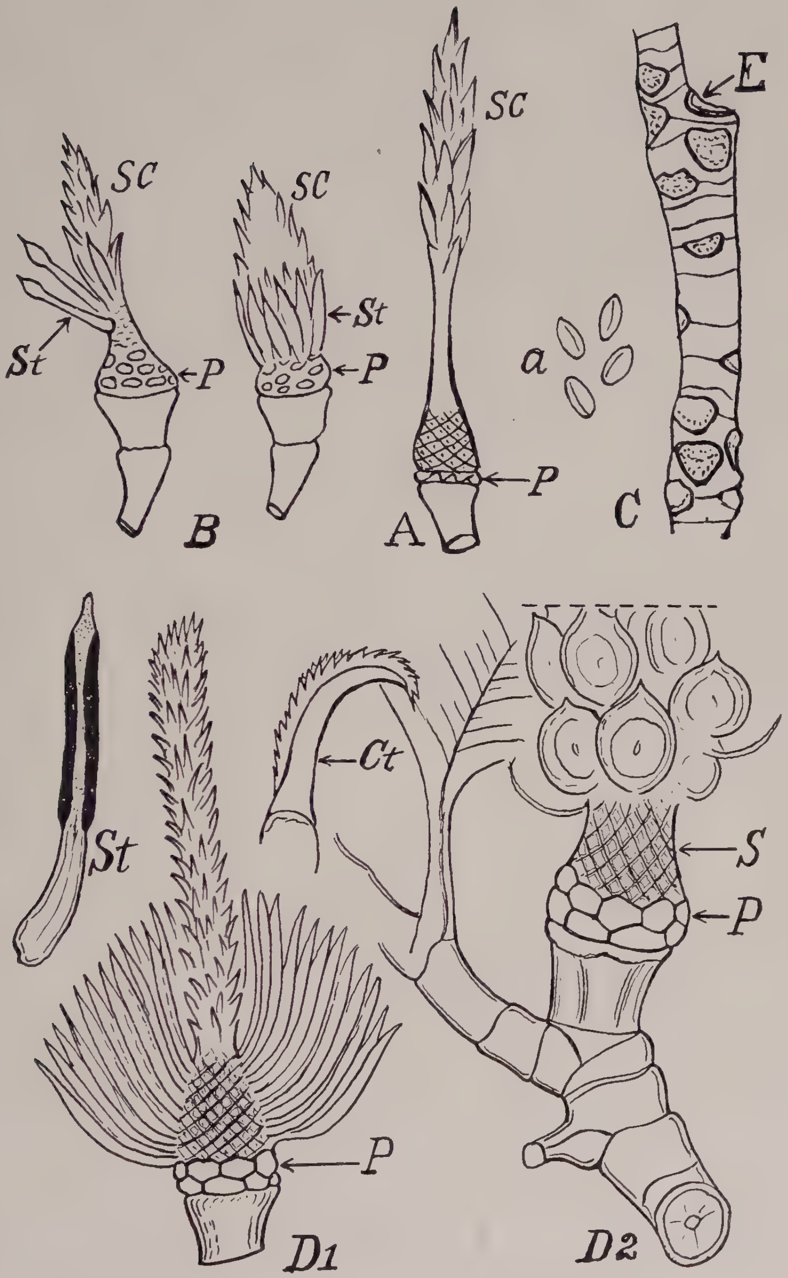

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AN EVALUATION OF THE STRUCTURAL EVIDENCES FOR GENETIC RELATIONSHIPS IN PLANTS: ALGAE[1]

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I have to-day the great honor of giving an account of the *algae* in this discussion, and I intend to show the bearing on the relationship of the *algae* of the known facts concerning their development and morphology. The limited time at my disposal will compel me to deal with but a few groups, but I shall endeavor to point out what are, in my opinion, the most important of the data to be considered in the determination of their relationship. And one of the most important of the newer points of view, as I take it, is the place in the life-cycle where the reduction-division occurs, this again being also connected with the alternation of generations which, I hold, must be regarded as a very important element in determining the relationship of plants.

The investigations made in the sphere of the brown algae, the great province of the Phaeophyceae, are of great general importance in this regard. The discovery in 1915 by the French algologist Sauvageau of the fecundation and the alternation of generations of the Laminariaceae—afterwards confirmed by numerous algologists, for example, Kylin (1916), Kuckuck (1916), Williams (1916), Yendo (1919), Printz (1922), and others—was a brilliant discovery, of equal significance with those which were made respecting the fecundation of brown and red algae in the middle of last century by the famous French algologists Thuret and Bornet, these last laying the foundation of the systematization of these groups of plants. French botany has once more, by these grand discoveries, upheld its lead in the sphere of algology. Up to the year 1915 the Laminariaceae, the largest of all brown algae, had been reckoned among plants which had only asexual reproduction by means of neutral swarm-spores. It was then shown that these give rise to a microscopically small sexual generation forming either antheridia or oogonia with one egg, and after fertilization there arises the well-known *Laminaria*-plant which was thus shown to be a sporophyte in a regular alternation of generations. Since Kylin in 1918 demonstrated the same course of development with respect to *Chorda filum* (L.) Stackh, and was also able to supply the additional information that the reduction division occurs in the sporangium at a place analogous to that in the oogonia and antheridia of the Fucaceae, the complete cytological and morphological life-cycle of the Laminariaceae has been demonstrated.

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany (Symposium: An evaluation of the structural evidences for genetic relationship), Ithaca, New York, Aug. 19, 1926.

Thus, the distinguishing feature of these plants, just as it is of ferns, is the dominance of the diploid sporophyte generation, the haploid generation being insignificant and transitory in their development.

The alternation of generations in the brown alga *Dictyosiphon foeniculaceus* (Huds.) Grev. also discovered by Sauvageau, in 1917, may be regarded as a variant of the *Laminaria*-type. This, too, has a microscopical sexual generation but, in contrast to that of the Laminariaceae, it is monoecious, that is, it forms male and female gametangia on the same individual. Besides, the gametes are isogametes, not morphologically differentiated into eggs and spermatozoids.

The general occurrence of an alternation of generations among the brown algae is confirmed in the work of Margery Knight (1923) on *Pylaiella litoralis* (L.) Kjellm. This alga occurs in a haploid (10 chromosomes!) monoecious, sexual generation which forms isogametes in plurilocular sporangia of the type normal to the Ectocarpaceae. After fusion these give rise to a diploid *Pylaiella*-alga morphologically like the haploid but forming unilocular sporangia in which the reduction takes place at the first division, so that all the spores become haploid. These afterwards form haploid *Pylaiella* individuals again.

Matters may be complicated, however, by the strong tendency of the *Pylaiella* gametes to an apogamous development, so that sexual generations are hereby easily regenerated. This is not in itself astonishing. It is more remarkable that Miss Knight found that diploid *Pylaiella* sporophytes can regenerate the same generation again owing to the fact that, together with the unilocular sporangia, in which the reduction division occurs it can also form plurilocular sporangia of exactly the same type as those of the sexual generation. These plurilocular organs of reproduction of the sporophytes produce, without a reduction division, swarmspores morphologically similar to those of the sexual generation. These are, however, diploid, and they never function as gametes but are developed directly and then, of course, as diploid individuals again.

The remarkable thing is thus that each generation can easily regenerate itself owing to the formation in the plurilocular organs of swarm cells which function as gametes only in the haploid generation. Both the haploid and diploid generations may thus form "plurilocular sporangia" of the same type, but only the haploid type can form gametes in these. The unilocular sporangium, on the other hand, which is the seat of the reduction division, is only found in the diploid generation.

Consequently, we know that the brown algae display several distinct types with respect to the degree of organization of the generations and the place of the reduction division in the life-cycle.

The first brown alga to be examined cytologically was *Fucus vesiculosus* L. whose reduction division was shown by Strasburger (1897), Farmer and Williams (1898), and Yamanouchi (1909) to occur at the division of the first oogonial and antheridial nucleus, consequently at the formation of the gametes before fecundation.

In *Fucus* there had thus been discovered a plant which, with respect to the position of the reduction division, almost completely corresponds to the animals,

at least the Metazoa, since only the gametes and their mother cells are haploid. Otherwise, the whole plant is diploid.

Almost contemporaneously with the discovery of the reduction division in *Fucus* another brown alga was investigated, namely, *Dictyota dichotoma* (Huds.) Lamour (Williams 1898, Mottier 1900). Here the circumstances proved to be very different. The gametes of *Dictyota* are formed without a reduction division but at fecundation there arises a *Dictyota* plant morphologically in complete agreement with the male and female individuals which formed the gametes, with the sole exception that it has only diploid nuclei in its tissues. This diploid plant does not form sexual organs but is propagated by spore formation alone. These spores are formed in tetrads within each sporangium and at their formation a reduction division occurs, with the result that the spores become haploid again. These haploid spores—as demonstrated by Hoyt—grow into sexual individuals again, so that the whole life cycle of the plant runs like a regular alternation of generations with regular alternation between haploid sex-individuals (gametophytes) and diploid spore-bearing individuals (sporophytes). This is in perfect agreement with what we have known, for example, in the ferns. The difference, however, is that both generations of *Dictyota* are morphologically alike, with the single exception of the structure of the nucleus, while both generations of the ferns are quite unlike each other.

Two strikingly different types of evolution of brown algae were now known with respect to the position of the reduction division in the life cycle. New ones were soon added, above all the remarkable case of *Cutleria multifida* (Sm.) Grev. (Yamanouchi 1909). As regards this plant it had long been known (Falkenberg 1879) that it had a pronounced alternation of generations, since an alga which had previously been reckoned as a proper genus, *Aglaozonia reptans* (Cr.) Kütz., proved to be the spore-forming generation of *Cutleria*. Now in 1909 a reduction division was demonstrated at spore-formation of *Aglaozonia*, quite as in *Dictyota*, and from these haploid spores there were formed haploid Cutlerias (♀ or ♂) which by typical divisions formed gametes. *Cutleria* thus proved to be of the same reduction division type as *Dictyota*, but its two generations, on the other hand, were very differently organized. The fact that the cycle of development in *Cutleria* might in certain cases be altered by the development of the gametes without fecundation (apogamy) was an important discovery which did not, however, in principle alter the cytological correspondence with *Dictyota*.

Four different types with respect to the alternation of generations and the position of the reduction division may thus be postulated: (1) The *Dictyota* type characterized by two independent, morphologically similar generations; the sexual forming gametes, by typical divisions (not reduction divisions); and the spore-producing generation concluded by a reduction division at spore formation. The majority of the brown algae probably belong here, that is, besides the Dictyotales also the Ectocarpales, Sphacelariales, and Tilopteridales. (2) The *Cutleria* type, like the foregoing, but with the spore-forming generation terminated by a reduction, morphologically unlike the sexual generation. (3) The *Laminaria* type, whose generations are likewise essentially different, so

different that the sexual one long escaped notice by reason of its insignificance. Consequently, the spore-forming generation completely dominated the life cycle. To this the *Dictyosiphon* type may be joined as a variant, characterized by a monoecious sexual generation and by isogamy, in contrast to the dioecious and oogamous sexual generation of the *Laminaria* type. This implies that *Dictyosiphon* is homosporous while the *Laminaria* type is physiologically, at any rate, heterosporous. (4) The *Fucus* type—the diploid generation completely dominant, with only the gametes of haploid nature; thus only one generation, morphologically speaking, is present.

Such a variety of types as the brown algae exhibit with respect to the relative degree of organization and the position of the reduction division in the life cycle of the different generations has not been found in any other group of plants. The seeming irregularity or lawlessness of the brown algae with respect to the position of reduction division was therefore a very striking phenomenon of which no explanation could at first be given. Certain systematists saw in this dissimilarity with respect to the alternation of generations and the position of the reduction division support for the assumption that the brown algae are not a systematically homogeneous group, but that they represent several different divisions not closely related to each other. This assumption would seem, however, to be too hasty, for reasons which will be hereafter adduced. We shall find, however, that the multitude of types can very easily be brought under one common heading so that they can be systematized in a natural way.

However, before postulating for the brown algae this natural system grounded on the extent of the different generations and culminating in the complete predominance of the diploid generation over the haploid—exactly as in the series mosses, ferns, gymnosperms, and angiosperms—we must first clear up the question whether the position of the reduction division in the life cycle of a plant is a secondary matter of no importance to the plant and, further, we must ascertain whether and why diploid organization has proved so superior.

The demonstration of the fact that the reduction division can occur at different points in the life-cycle of the plant and, further, that in the same systematic group it has been shifted, soon led some botanists, as Oltmanns (1905) and Goebel (1913), to pronounce the opinion that it was a matter of indifference and quite insignificant to the plant and its alternation of generations where this reduction division occurred. When, by fecundation, a doubling of the number of chromosomes has occurred, this must be "compensated" by a reduction division, for the avoidance of an infinite doubling of chromosomes; but when and where this reduction might occur was indifferent to the plant. The dissimilarities with respect to the position of the reduction division in plants was the reason why no uniform view of the phenomenon could be obtained. So the position of the reduction division was declared to be quite without significance. It was merely presented as an interesting but otherwise unimportant fact that plants, in this respect, displayed various types, while animals displayed one uniform type.

This view of the matter, however, is in my opinion quite wrong. The nature of the reduction division, that of being able to recombine the chromosomes

in the daughter nuclei, must undoubtedly be regarded as of great significance when seen in the light of Mendelism. Those who regard the position of the reduction division as a subordinate matter are attaching too much importance to its significance for the "reconstruction" of the *number* of chromosomes doubled by fecundation, but the great significance of this division consists in its possibilities for preparing the recombination of chromosomes (= "factors" or "characters") in different nuclei. In a certain measure it may thus be regarded as the final act and goal of fecundation. Exactly similar diploid nuclei, by a reduction division, may give rise to daughter-nuclei differing considerably, as chance combines the chromosomes in these daughter-nuclei. When the chromosome number of a plant is known, it may easily be calculated how many combinations are possible. At every reduction division in a nucleus, in plants at any rate, only two possibilities at the most can be realized. This is because in all the plants whose reduction divisions have been investigated the first division of the nucleus is heterotypical while the second is an ordinary division. Thus, in a tetrad, of the four nuclei formed there will always be two alike with regard to the arrangement of the chromosomes. It is for that reason easy to calculate the minimum number of nuclei undergoing reduction division in order to obtain all conceivable combinations of chromosomes. If the haploid chromosome number of an organism is a and its diploid consequently $2a$, the number $2a$ indicates the number of possible different haploid nucleus-combinations; and the number $2a$–1, the theoretically minimal number of nuclei undergoing reduction divisions needed in order that all these shall be able to be realized. Now these numbers are the same as those characterizing the Mendelian character pairs. For example, a plant with 10 chromosomes in the haploid nucleus, and consequently 20 in the diploid, can by reduction divisions combine them so that 1024 ($=2^{10}$) different haploid nuclei can be formed, and for this at least 512 (2^{10-1}) different reduction divisions are required.

Thus, in cases where diploid nuclei with exactly the same arrangement of chromosomes are divided by a reduction mitosis in different ways, it is fairly evident that when a fecundation is at once followed by a reduction division, that is, one fecundation is followed by only one reduction division, then never more than two chromosome combination possibilities can be realized. On the other hand, when a more or less highly developed diploid phase or an independent diploid stage (sporophyte) forms numerous spore-mother-cells each undergoing reduction division, that is, one fecundation is compensated by many reduction divisions, then numerous combination possibilities can be realized, increasing in number in proportion to the number of reduction divisions which occur. This cannot be an insignificant matter. From this it follows that it cannot be indifferent when, where, and how the reduction division occurs in the life cycle of a plant.

There are, in the main, two different cases in the vegetable kingdom: the fertilized diploid nucleus is either divided immediately (thus one fertilization and one reduction division) or else the reduction division is delayed and there is formed a diploid organization or sporophyte, wherewith one fertilization is

always counterbalanced by several reduction divisions. No type is known in which the reduction division is delayed, a diploid sporophyte is formed, and in which a single reduction division follows and compensates for fertilization. It is not likely to be found although, of course, it is theoretically conceivable.

A survey thus shows that a delay of the reduction division implies that for one fertilization there always come many reduction divisions. In diploid organisms there is the possibility that fertilization becomes, so to speak, better utilized, so that many reduction divisions are attained—more than in the case of a haploid organism with the zygote as the only diploid stage. Diploid organisms, or organisms with at least one many-celled diploid stage inserted into the life-cycle, must therefore on purely theoretical grounds be deemed more highly organized than purely haploid organisms where the reduction division immediately follows fecundation.

It is thus manifest that "cytological generations" are of fundamental importance, and often more fundamental than morphologically differentiated individuals. These are by no means "purely academic conceptions as a concession to the mysteries of Cytology"! as Church (Thalassiophyta and the subaerial Transmigration. Botanical Memoirs 3, pp. 61 [note]. Oxford, 1919) seems to believe.

It is manifest that the multiformity of organisms in nature cannot be explained solely by recombinations, "hybridizations," but, on the other hand, it is certainly proved that one method of origin of new genotypes is hybridization, which is thus one cause of the multiformity in nature. New "species" may arise from crossing, and modern plant breeders work entirely with crossings on the principle that individuals possessing the properties which it is desired to have combined are experimentally crossed with each other. If the desired combination is a rare one with very small prospects of realization, the plant breeder is obliged time after time to repeat his crossings until, finally, the desired combination really appears. The more crossings, the greater the probability that the thing desired will be attained.

All our cultivated plants are now, as seed plants, diploid organisms. A consequence of this is that every individual supplies pollen and embryo-sacs of many different kinds whereby the chances are increased that the desired combinations will be realized. Imagine that plant breeders worked with haploid organisms instead. All the pollen formed from the same individual would then be genotypically identical. We can easily see that far more individuals would be needed and far more crossings made in order that all the possible combinations of chromosomes might be realized, since every individual is only the bearer of one such combination. The diploid plant, on the other hand, without taking up more room and without having a greater need of nutriment than the haploid, is the bearer of several such possible combinations. Supposing that unlimited space were at the disposal of a certain species in nature and, further, that all fertilization combinations could be easily attained, it would then, perhaps, be indifferent whether an organism were haploid or diploid. The possibilities of fertilization in nature are, however, strictly limited, so that only a small fraction of all gametes really attain their end. With respect to all plant organs we find

that they are thus far purposefully organized that they attain the greatest effect with the least use of material. This seems to me to be the case also with the diploid plant as a whole as regards its capacity to form offspring of a great diversity of chromosome combinations.

For, when plants are examined in order to ascertain whether they are of haploid or diploid organization, we cannot escape noticing the remarkable fact that the classes regarded by taxonomists, often on other grounds, as the highest are, as a rule, diploid, while the purely haploid organisms are met with among those classes of plants which have long been regarded as primitive. Then there are a whole lot of plant types which occupy, as it were, a middle position between haploidi and diploidi, an approximately equal portion of the life cycle falling within both these phases. This is the case, for instance, with plants having decided morphological alternation of generations. We also find plant classes where certain sub-groups are haploid, others diploid.

Among plants with predominantly or exclusively haploid phase we find, for example, among the algae, the Conjugatae and probably the bulk of the Chlorophyceae which are plants of fairly low standing, while Gymnosperms and Angiosperms, that is, the highest, belong to the predominantly diploid categories. Even a cursory examination thus shows that types with one fertilization and one reduction division have not raised themselves to the same grade of organization as types with one fertilization and many reduction divisions, that is, reduction division in many nuclei.

Finding then, as we do, that the most highly organized forms of the vegetable kingdom are exactly those where more or less enduring diploid organizations have arisen, and finding, further, on purely theoretical grounds, that this must be deemed an advantageous organization from the point of view that a greater wealth of forms is made possible, we must regard the diploid organization as a favorable contrivance for the organism or species, apart from the question of what physiological factors brought it about. Useless types, unfit for life, are indeed produced by Nature, but they are soon weeded out by selection. This does not, however, seem to be the case with plants having the diploid organization; for they have proved decidedly more fit than the purely haploid ones.

Most botanists are, no doubt, agreed that haploid organization must be deemed more primitive than diploid. For we are bound to regard fertilization and the fusion of gametes and nuclei as primary, while the reduction division, as a consequence of nuclear fusion, is secondary. In primitive forms there is often no morphological difference between asexual spores and gametes. The classical example of a primitive type of fertilization in the vegetable kingdom is *Ulothrix zonata* (Web. et Mohr) Kütz. It forms swarmers (zoospores) provided with cilia which are developed directly. Other similar ciliated swarmers prove to be gametes which, by copulation, give rise to a zygote which (probably) undergoes immediately a reduction division, and produces new haploid swarmers. The sexuality of the gametes is here, however, little marked, as they may also be developed without fertilization (parthenogenetically) into new individuals. Cytologically this is easily explained, in so far as both plant and swarmers

(whether gametes or not) are haploid. It is therefore tempting to assume that sexually differentiated gametes have arisen from asexual swarmers (zoospores) of flagellate type.

The assumption that an organism which is now purely diploid, such as *Fucus*, is primitive (that is, has no past haploid generation—except the sperm, viewed as a regressive flagellate, which may have directly built up the *Fucus* plant—as Church seems to believe, (Journ. Bot. **63**: 1925) meets with great difficulties. In that case, the reduction division must be assumed to be the primary phenomenon, and one of the functions of fertilization is to accomplish a return to the supposedly primitive condition. This assumption is highly improbable, while, on the other hand, it is not so difficult to understand that a swarmer which is, perhaps, otherwise neutral might unite with another prior to the further development whereby the first fertilization might be inaugurated. We shall find, too, that this view of the relation of fertilization and the reduction divison to one another leads to a very natural system.

Ever since the structure of the nucleus in the greater groups was appreciated there have been guesses that diploid organization may be advantageous to the plant. Indeed, it soon became evident that the diploid organization was completely predominant among the higher plants.

The idea was first put forward that the presence of diploid nuclei causes a stronger cellular structure generally. When we first became acquainted with the frail haploid fern protallium and compared it with the robust diploid fern plant, it was tempting to explain this difference by referring to the structure of the nucleus. This assumption was supported by certain cytological phenomena, for example, that in such genera as *Oenothera* (gigas), *Crepis*, etc., the more robust species have a larger number of chromosomes than the frailer ones. The discovery of the condition pertaining in *Dictyota*, with its two generations exactly similar morphologically, was the deciding factor in the rejection of this view.

Another view which sought to give an explanation of the predominance of the diploid organization is very closely connected with the problem of the alternation of generations in general. Starting from the fact that in mosses and ferns the haploid generation appears in several respects organized for an aquatic life (this being most noticeable in the formation of spermatozoids, which require water in order to reach the archegonia) while the diploid generation in its general organization appears to be a more decided land plant, with spores which are spread by the wind, etc., Bower (1890) and Wettstein (1903) assumed that the alternation of generations had originated in the transmigration of the plant world from water to land. By this view the diploid generation is a new formation, organized for land life and formed, according to Bower, by a "progressive sterilization" of the sporogenous tissue which arises from the zygote. In proportion as plants adjusted themselves for life on land, the diploid generation became the dominant one, while the haploid generation, fitted for an aquatic life, was reduced. The first cases of the alternation of generations cytologically studied in marine algae yielded some support to this opinion.

Both in *Dictyota* and in the Florideae, haploid and diploid generations were found morphologically alike. Explanation was sought in the fact that both generations lived in the sea. The peculiar reduction of the haploid generation in favor of the diploid in the series mosses-ferns-seed-bearing plants was therefore something new. The dominance of the diploid generation thus involved a migration theory.

This migration theory was, however, seriously marred by Sauvageau's discovery of the alternation of generations in the Laminariaceae. Through this discovery the formerly isolated groups of brown algae could be joined together into a uniform system in a very natural way, and the gulf between the Fucaceae and the Laminariaceae could be bridged over.

The simpler and least differentiated brown algae, like *Pylaiella* of the Ectocarpales, have a marked alternation of generations, with the reduction division located in the unilocular sporangia of the diploid generation. The unilocular sporangium of the diploid generation has no morphological parallel in the haploid. Here we find: *Pylaiella* (monoecious isogamous), and *Dictyota* (dioecious, heterogamous, homothallic). *Cutleria* has in principle the same development but the generations are morphologically considerably different since the diploid generation is of a more primitive stage.

The Laminariaceae are naturally linked with these last by the newly-discovered alternation of generations but the diploid generation dominates in its development, the haploid generation being insignificant, indeed so insignificant that it is sometimes reduced to merely one or two vegetative cells which form the gametangium. The striking feature of the whole type is the overwhelming domination of the diploid generation in the life cycle.

The Fucaceae may now be attached to these without difficulty, thus the discovery of the alternation of generations in the Laminariaceae has led to the bridging over of the chasm between this group and the Fucaceae. As just now mentioned certain Laminariaceae form such insignificant haploid generations that they are limited to a single cell. The diploid generation thus forms a haploid spore which divides only once, into one vegetative cell and one fertile one, the latter becoming the gamete which is fertilized. Should this single vegetative cell now fall out of the cycle, and the fertilization still be preserved, the result will be that the spore itself will become a gamete and the plant will be entirely devoid of the haploid generation. The Fucaceae type has evidently done this, for the *Fucus* plant is similar to and homologous with the *Laminaria* sporophyte and not with the *Laminaria* gametophyte. *Laminaria* is morphologically homosporous, but *Fucus* must be assumed to be descended from a heterosporous type, as it forms morphologically different gametes (here = spores), but this difference does not influence the view expressed, since we know that homospory and heterospory often occur within the same systematic groups, as in the ferns. The fact that this homology was not instantly seen was mainly because *Fucus* is a sex-plant and since the alternation of generations has long been defined (although not quite correctly) as an alternation between sexual and asexual generations, this homology met with opposition—compare Scott (1896), Fritsch

(1916), etc. We have here a typical example of the power of language over thought. The view that the diploid sporophyte generation, in an alternation of generations, is asexual cannot, however, be upheld. Even the heterospory apparent in the sporophytes of ferns is essentially a sex-differentiation into male (micro-) and female (macro-) spores. There are, to be sure, in ferns no sporophytes which form only one or the other kind of spores, thus no one-sex sporophytes, but the type is met with even in conifers, for example, in the yew and juniper, both of which are dioecous trees, and in the angiosperms it becomes common.

The diploid Fucus must then be viewed as the surviving diploid generation in an alternation of generations whose haploid generation has quite disappeared, the haploid spores (the sole haploid feature in the whole life cycle) having become gametes. From one point of view the *Fucus* gametes are spores, in my opinion. These are, then, the final products of a diploid generation, arising from a reduction division which produces no haploid sexual generation, becoming gametes directly, that is, eggs and spermatozoids.

Interpreted in this way, the whole series of brown algae becomes clear. We have to do here with algae which in their simpler forms (*Pylaiella*) show an alternation of generations with fairly evenly balanced haploid and diploid generations. In the Laminariaceae (and *Dictyosiphon*) the diploid generation has the upper hand entirely, and finally in the Fucaceae, this last alone comprises the whole plant.

It must now be borne in mind that the position of the reduction division is the same within the whole of this series, that is, always at the time of spore formation in the diploid generation,—this being so in *Pylaiella*, *Dictyota*, *Laminaria*, and *Fucus*. There has been no displacement within this group, although there would seem to have been, since in *Fucus* the reduction division occurs before the formation of the gametes, while in the others it occurs long after fertilization, at the end of the diploid generation. Since *Fucus* must be homologous with this diploid generation—concluded by the formation of spores which become gametes—it will be seen that the position of the reduction division is not disturbed. Within this series the haploid generation has been more and more reduced, at last disappearing. This view gives a natural explanation of the life cycle and thus of the classification of the brown algae.

What has happened within the series of brown algae is, however, exactly the same as what happened within the type-series mosses-ferns-gymnosperms, and angiosperms and was first cleared up by the brilliant genius of Hofmeister. Here too the progressive predominance of the diploid generation is conspicuous. Thus, within very different groups of plants we meet exactly the same tendency—the final predominance of the diploid generation over the haploid.

I have already endeavored to show that the diploid organization must be deemed superior to the haploid. But in an alternation of generations how are we to account for this reduction of the haploid generation to the point of disappearance of all structures except the gametes? A biological explanation is that otherwise the generation becomes superflous. The purpose of the haploid

generation is merely to produce many gametes, all genotypically alike, but this generation as such becomes superfluous when the reduction division is associated with spore formation, that is, with the formation of propagative bodies becoming gametes directly, as in the case of *Fucus*. The elimination of the haploid generation means a high degree of simplification in the life cycle of a plant, without lessening the number of fertilizations (=recombinations of characters); instead, the reduction divisions are increased and combined with the formation of the propagative bodies.

The classification of the brown algae (Phaeophyceae) may thus be based on the results obtained by such research, accordingly, they are divided into the following groups:

I. **Phaeosporales.** Alternation of generations consists of morphologically similar generations; plurilocular organs form gametes; unilocular organs are the seat of the reduction division. The swarm cells of the plurilocular organs have a great tendency towards apogamy. Oogamy is not typically differentiated but a tendency thereto can be traced. With respect to the manner of vegetative growth the group can be fittingly divided into two series:

(1) *Ectocarpales*, generally with intercalary growth.

(2) *Sphacelariales*, with the growth of the thallus by an apical cell.

II **Cutleriales.** Both generations alike (*Zanardinia*), or different (*Cutleria*); very evident tendency to oogamy.

III **Dictyosiphonales.** Alternation of morphologically dissimilar generations, the sporophyte exceeding the gametophyte in size: gametophyte monoecious, isogamous: transition type from Ectocarpales to Laminariales; the limits of the group still uncertain.

IV **Tilopteridales.** Probably a morphological alternation of similar generations; oogamy apparent; sporophyte producing monosporangia, probably with incomplete divisions of the spores. The order is a transition type from Ectocarpales to the next.

V **Dictyotales.** With morphological alternation of similar generations; gametophyte oogamous; sporophyte bearing sporangium with only 4 spores (= tetraspores).

VI **Laminariales.** A morphological alternation of very dissimilar generations; gametophyte very small, oogamous; sporophyte bearing sporangium with many spores (morophologically isospores, physiologically heterospores).

VII **Fucales.** Morphological alternation (probably) reduced, only cytological indications; oogamous; the "alga" diploid and homologous with the sporophyte in the Laminariales. This is the climax-family of the brown algae, perfectly analogous with the most highly developed Phanerogams in respect of alternation.

We thus find that the present brown algae can, generally speaking, be grouped into a few natural orders characterized not only by the structure and differentiation of the organs of reproduction but, above all, by the degree and extent of evolution attained by the different phases in the alternation of generations.

The theory of the alternation of generations has thus proved highly valuable in classification and although dealing here with certain marine plants only, the

results are perfectly analogous to the case of the higher land-plants (mosses, ferns, gymnosperms, and angiosperms). This seems to me to prove conclusively that the alternation of generations has, of itself, nothing to do with the migration of the plant world to the land. The relative evolution of the two generations is regulated by other factors intimately connected with the proper nature and significance of fertilization itself and, above all, with the significance of the reduction division as the climax and goal of the act of fertilization.

This result of research into the taxonomy of the brown algae seems to me of such weight and significance that the effects must have influence far beyond the boundaries of algology proper.

We now pass on to the red algae, Rhodophyceae or Florideae, and we find that here too, our knowledge has been considerably extended these later years. Yet, it can hardly be asserted that the plant group in question has had its classification reversed or re-made to the same extent as the Phaeophyceae.

The two Agardhs—father and son—laid the foundation for the early taxonomy of the red algae by paying attention to the structure of the cystocarp. Upon this, further work was done in the course of time. When, later on, fertilization was discovered in these algae by the eminent French algologists Thuret and Bornet, in the sixties, it was natural that still greater importance from the systematic point of view should be attached to the cystocarps and the remarkable organization arrangements, the auxiliary cells, etc., which are connected therewith. The man who laid the real foundation of the modern Floridean system was, however, the famous German algologist at Greifswald, Fr. Schmitz, by his investigations into the origin and early development of the cystocarp. But it was soon apparent that respecting the red algae also cytological research could yield important aid, even to taxonomy. In 1906, when the first cytological investigaion on a red alga was published, namely, the fine work of Yamanouchi on *Polysiphonia violacea* in the Botanical Gazette, a new epoch was created in the history of Rhodophycean research. By this the reduction division was shown to occur in the tetrasporangium. The tetraspores of the red algae are, as a rule, formed on special individuals, like sex-individuals. These latter are, of course, haploid and the tetrasporic individuals diploid, but matters are complicated in the red algae by the fact that after the fertilization of the female organ (that is, the carpogonium) the tetrasporic generation is not directly developed, but there is first formed a richly ramified system of sporogenous filaments which form a special kind of propagative bodies, the so-called carpospores, which only later on form tetrasporic individuals. The whole of this system of sporogenous filaments, together with a cover eventually surrounding them, is termed a cystocarp in the red algae. The sporogenous filaments of the cystocarp, as also the carpospores, were found to be diploid in *Polysiphonia*. The haploid phase of such a red algae consequently consists of the sex plant; but the diploid phase consists partly of the sporogenous filaments of the cystocarp, which always live in organic connection with the sex plant, and partly of tetrasporic individuals.

Now it had long been known that a number of red algae belonging to the

group Nemalionales never formed any tetraspores. They too, however, exhibited fertilization, but as they did not form tetraspores, the reduction division must take place somewhere else in the life cycle. But where?

In *Scinaia furcellata* (Turn.) Biv., however, I succeeded (Svedelius 1915) in showing that the reduction division in the non-tetrasporic Florideae occurs immediately after fertilization. The discovery was confirmed as to *Nemalion* and *Batrachospermum* (Kylin 1916, 1917). Owing to this fact the sporogenous filaments of the cystocarp in these Florideae become haploid, like the carpospores, and no special tetrasporic individuals occur. The explanation of their absence lies in the position of the reduction division. Red algae of this type of development appear in nature with but a single kind of individual—haploid sex individuals. I have therefore called them haplobiontic, in contrast to the other type which I call diplobiontic, because they occur with two kinds of individuals alternating with each other—haploid sex individuals and diploid spore-forming individuals.

In the Florideae we have thus found a natural, uniform group of plants where the reduction division takes place at quite different places in the life cycle, with the result that organs which are undoubtedly, from the morphological point of view, homologous—the sporogenous filaments of the cystocarp—may be either haploid or diploid.

Differing, as they do, in their life history, how are these Floridean types to be accounted for? To me the simplest explanation is to assume that the reduction division has been delayed. For several reasons the haplobiontic Florideae must certainly be regarded as the more primitive. When the reduction division, for some reason, does not occur immediately after fertilization, as in *Scinaia*, but is delayed, the consequence must be that the sporogenous filaments and the carpospores of the cystocarp become diploid. The diploid carpospores grow into individuals like sex individuals excepting that they are diploid. Such diploid individuals do not form sex organs, but they do form sporangia and a reduction division occurs at spore formation, so that haploid sex individuals can once more be formed from the spores.

By assuming a delay in the reduction division we obtain without difficulty an explanation of the two different types of Florideae with respect to the position of the reduction division in the life cycle. This assumption is, no doubt, highly antagonistic to the current view which prefers to see in the nuclear fusion and in the reduction division the two fixed points in the ontogenetic development of organisms, around which, especially, cluster the possibilities of evolutionary change.

There are not wanting, however, pronouncements from another quarter which have a relation to the problem of the delay of the reduction division in plants.

Ernst (1908) sought to explain the irregularities or, perhaps better, the different types of behavior of the embryosac of the phanerogams as being due to a brief displacement of the position of the reduction division in the life cycle. This view found an enthusiastic supporter in Buder (1916) while embryologists in general were sceptical. Without taking any position on this question it seems to

me of interest in this connection to point out that, as regards other plants also, the view has been held that the reduction division can be delayed.

The delay of the reduction division in the red algae has brought it about that there are always two different types, haplobiontic and diplobiontic. Following on what I stated above, in connection with the brown algae, respecting the nature and significance of the reduction division in general, it is evident that all the nuclei in the carpospores in the same cystocarp of the haplobiontic red algae are, with respect to their chromosomes, equal all originating from the only surviving nucleus of one single tetrad of which the other three nuclei have degenerated; while tetraspore formation in the diplobiontic red algae occurring, as it does, at many different parts of the plant, affords a far greater opportunity for varying the chromosome composition (=factors). Therefore, the diplobiontic red algae are better organized than the haplobiontic by reason of the greater possibilities for the dissociation of characters. Accordingly, I do not hesitate to regard haplobiontic Florideae as more primitive. The question may now be raised: How does this cytological classification agree with the old system of the red algae?

As far as known, all proved haplobionts are to be found in Schmitz' group Nemalionales, which, in its entirety, may be pretty certainly characterized as haplobiontic. This demonstration alone seems to me to imply a great step forward systematically. The generally simpler organization of the group Nemalionales appears also to confirm the fact that the more primitive organization of this type with respect to the reduction division has a correspondingly simpler morphology and less wealth of forms both in families and in species.

The diplobiontic red algae include the largest families and those richest in forms as well as the most highly differentiated types. All the other groups postulated by Schmitz should be found here: Cryptonemiales, Gigantinales, etc. On the other hand, it is not impossible that a haplobiontic type might be able to arise by reduction, that is, by the elimination of fertilization, or in some other manner whereby tetraspore formation is eliminated. In fact, certain observations indicate that such a thing may really occur. (Compare Kylin's paper on *Bonnemaisonia* and Kuckuck's on *Platoma*.) When such cases have undergone proper cytological investigation, the question of the taxonomic significance of haplobiontic and diplobiontic organization must be taken into consideration anew.

At present it may be asserted, at any rate, that cytological research on the position of the reduction division in the red algae has thrown quite a new light on the whole classification of this group.

I cannot, of course, deal with the numerous special articles on the group which have considerably extended our knowledge of the details of the organization of the cystocarp, the more so as they do not seem to have disturbed the principles already laid down as a ground-work for the systematization of the red algae.

Finally, it is essential to say a few words about the green algae, the Chlorophyceae and the Conjugatae. Can it be said that cytological research has also

influenced our view of their taxonomy? Most investigations in these groups indicate that fertilization is immediately followed by the reduction division, this being so in *Coleochaete*, examined by Allen as far back as 1905, and in *Spirogyra*, examined by Tröndle in 1911, etc. From this it seems probable that the green algae in general are to be viewed as haplobiontic and no diploid generation, except the zygote, seems to have attained development. But in this case, also, cytological research in quite recent times has supplied suggestions the taxonomic value of which it is still too early to estimate accurately. In a paper recently published in the Proceedings of the Linn. Soc. of New South Wales an Australian algologist, May Williams, has shown that the almost cosmopolitan siphonaceous alga *Codium tomentosum* has a diploid organization. The reduction division occurs in the great unilocular sporangia, while the spores are haploid, functioning like gametes. The gametes are the sole haploid element in the whole life cycle and the alternation of generations is thus purely cytological. We thus meet here, even among the Chlorophyceae, types which cytologically correspond to the *Fucus* type among the Phaeophyceae. How this may influence our view of the system of the green algae it is, as yet, impossible to say. It is certain, however, that if the observation is positively confirmed, and more such types are met with among the Chlorophyceae, there is bound to come in time an essentially changed view of the entire phylogenetic connection between the different classes of the green algae and thus of their classification.

To summarize what I have now dealt with in general outline about our present view of the natural relationship and development of the larger groups of algae, I desire to emphasize my opinion that modern algal taxonomy cannot avoid paying careful attention to the attainments of cytological research, especially as regards the establishment of the position of the reduction division in the life cycle. Just as classification has long been based on the structure of the sex organs, it ought likewise in future not to neglect the place of the organs in which the reduction division occurs, for the reduction division is not only the complement but also actually the final act or goal of fertilization, and the various types in respect to the position of the reduction division in the life cycle also reflect the different grades of development which the algae have undergone.

Exactly as the higher land plants—mosses, ferns, gymnosperms, and angiosperms—show that the center of gravity, so to speak, is removed from the haploid to the diploid stage, in like manner we see now—thanks to the results of cytological research—that within the plant world of the oceans, also, exactly the same course of development may be traced. This seems to me not the least important result of the researches of the last few decades into the development and taxonomy of the algae.

AN EVALUATION OF THE STRUCTURAL EVIDENCE FOR GENETICAL RELATIONSHIPS IN PLANTS

Some Evidence from Vascular Plants[1]

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All attempts to arrange plants in a natural, or genetic, sequence have been based upon the evidence afforded by similarity of structures. Morphologists of the older school believe that most of this evidence, especially that afforded by embryology, is reliable; modern morphologists believe that some of the evidence, but not so much of it, is due to genetical relationship; and there are physiologists and ecologists who would go still farther and claim that the external form and internal structure of a plant are due, almost entirely, to environment.

Heredity and environment are the factors which determine the form, structure, and life history of a plant. Morphologists have over-emphasized the influence of heredity, while physiologists and ecologists have over-emphasized the influence of environment.

It is becoming more and more evident that environment is a potent factor and that some of the things which have been attributed to heredity may be due to environment; but, as an acute observer remarked some 2000 years ago, you do not gather figs from thistles; and very probably, if there had been constant experimentation from that time up to the present, not a single member of the fig family could have been transferred to the sunflower family. Heredity cannot be disregarded, but environment must be recognized as a powerful factor.

It is highly desirable that the rising generation of morphologists should have enough physiology and ecology for effective research in experimental morphology; and that physiologists and ecologists should have enough morphology for effective experimental work in their own fields. Such researches would not only facilitate an evaluation of the structural evidences for genetical relationships in plants, and be of the utmost importance in all phases of morphology, but they would show what structures can be modified and how far structures can be made to deviate from their usual path. The apple, pear, and quince resemble each other rather closely and we believe that their structural similarities are due to genetical relationship: in the same way, the cherry, peach, and plum have a genetical relationship which is indicated by similar structures. In each group, the three members mentioned have come from a common ancestor in the remote past;

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany (Symposium: An evaluation of the structural evidences for genetic relationship), Ithaca, New York, Aug. 19, 1926.

and, farther back, these two common ancestors have come from some still more remote ancestor.

Structures are modified in varying degrees and the amount of modification indicates the degree of relationship. The morphologist stresses mutation, smaller variations, hybridizing, and natural selection in accounting for changes, and he is too likely to overlook the influence of soil, climate, and other conditions.

A submerged leaf of a water plant may differ decidedly from an aerial leaf of the same individual; and it is well known that the aquatic form of *Polygonum amphibium* differs so much from the land form, both in habit and in structure, that the two forms were once mistaken for distinct species. Just how far the capacity for such immediate responses is inheritable, we do not know. Would *Proserpinaca palustris*, grown in a mesophytic habitat for a thousand generations, lose the capacity for producing dissected leaves when grown in the water? Again, we do not know.

Nearly all botanists believe that the land flora came from an aquatic ancestry, and no one doubts that algae preceded vascular plants. When algae transmigrated to the land, it became necessary to develop protective, conducting and supporting structures which had not been produced while the plant was surrounded by water, with no need for protection, conduction or mechanical support. The theories of Church,[2] as expressed in his Thallassiophyta, and the views of Scott[3] as set forth in his Ancient Plants and in the third edition of his Studies in Fossil Botany, and also the views of Seward[4] are very suggestive.

At first, we may suppose, the conducting cells were merely somewhat elongated. Later, a conducting strand appeared and, still later, a vascular system. The development of conducting cells was a response to surroundings, and the conducting structures at some period, became hereditary. By mutation, by smaller variations, by natural selection, or in some other way, an efficient conducting system was developed.

Later, more algae may have transmigrated to the land and having the same conditions to contend with, may have made similar responses and developed a conducting system which became efficient and assumed many of the characters of the previous transmigrants.

It has been assumed that two conducting systems of this sort, closely resembling each other, owe their similarity to genetical relationship. Paleobotanists are often entirely dependent upon such evidence because the wood may be the only part of the plant preserved; but morphologists, especially anatomists, lay no less stress upon the evidence of vascular anatomy. And the resemblances may be due to heredity, but it is very possible that some of the structures may be due to similar environment and that plants may be very much alike without being even remotely related to each other. Thus it would be possible for plants like *Rhynia*, *Hornea*, and *Asteroxylon* to have developed inde-

[2] Church, A. H., Thalassiophyta and the Subaerial Transmigration. Botanical Memoirs, No. 3. Oxford University Press, 1919.

[3] Scott, D. H., Studies in Fossil Botany, 3rd. Ed. A. and C. Black: London, 1923.

[4] Seward, A. C., Fossil Plants. Vol. 3, 1917; Vol. 4, 1919. Cambridge University Press.

pendently of each other and even from different algae; but it seems more probable that, at least, the two species of *Rhynia* are genetically related and that there is a relationship of some sort between *Rhynia* and *Hornea*.

It is doubtful whether any two genera of the living heterosporous Pteridophytes are closely related to each other, with the possible exception of *Pilularia* and *Marsilia;* but they are all heterosporous, and their heterospory has been achieved in the same way—by the disorganization of potential megaspores which have been absorbed by the growing megaspores. We should be slow to believe that *Azolla* has been derived from *Salvinia* by direct descent, or that *Salvinia* has come from *Azolla;* it is easier to believe that similar conditions and a general tendency in evolution have produced heterospory independently in the two genera. Heterospory would seem to be the goal toward which a general tendency of evolution is urging all the vascular plants.

The Cycadofilicales bear a very close resemblance to the ferns, so close that they were mistaken for ferns until their seeds were discovered. Could it be possible that transmigrants, coming upon the land at different periods, have developed into these two groups independently? Since the two groups are associated geographically and edaphically, the conditions they had to meet must have been somewhat similar. The demands of protection, absorption, conduction, and assimilation would probably have resulted in the production of roots, stems, and leaves; but we doubt whether these organs, developed independently, would be so similar that the two groups would be mistaken for each other. It is easier to believe that the Cycadofilicales have come from the Filicales by direct descent. We believe it to be a fundamental of development that homospory must precede heterospory. The tendency toward heterospory is so strong that this condition has been achieved independently by various lines of vascular plants.

The seed is the final stage in the development of heterospory. We can imagine cases in which it would be difficult or even impossible to draw a line between a heterosporous Pteridophyte and a seed plant. If the retention of the megaspore makes the sporangium, with its contained megaspore, a seed; while a sporangium which sheds its megaspore, even at an advanced stage in development, has not yet reached the seed condition, a single individual might be partly fern and partly seed plant. Such a condition sometimes occurs in *Selaginella*, and may have been rather frequent as the early seed plants were evolving from heterosporous Pteridophytes. The two groups, at the transition stage would be separated by artificial definitions rather than by facts.

During the period while the advanced megasporangium was developing into the true seed stage, it would not be surprising to find the leaf remaining at the fern level. In very recent times, apples have developed so that numerous varieties, of very different aspect, have arisen, while the leaves remained about the same.

In vascular anatomy, the Cycadofilicales are more advanced than the ferns with which they were associated. To cite a single feature, circular pits are very characteristic of the xylem of the higher seed plants while a scalariform marking

is equally characteristic of ferns. The known Cycadofilicales have quite generally progressed beyond the scalariform stage, but we should expect to find it in their seedlings; for even the living cycads pass through a scalariform stage and *Stangeria* does not pass beyond it, except in certain tissues. This genus also retains a very fern-like leaf. The case of *Stangeria* is particularly instructive, for it shows that a very advanced seed condition may be attained while the leaf and vascular anatomy remain near the fern level. Evolution does not progress at the same rate in all features of a plant; and so we should not be surprised if we find stelar structures advancing, while the leaf and general topography of the plant remain more or less stationary. The Cycadofilicales might have retained the fern leaf, while the stelar structures advanced and the megasporangium passed beyond the heterosporous fern stage to the true seed condition. Plants bearing seeds and those which had almost, but not quite, reached the seed condition, would naturally resemble each other so closely that they could not be distinguished by vegetative characters; and the resemblance would be due to genetical relationship.

Do not accuse us of putting up a straw man, for no less an authority than Scott, the great paleobotanist, recently made that statement that the "Cycadofilicales now appear as a totally distinct line, parallel in certain respects to the true ferns, but nowhere joining them, unless it be in some common thalloid source, about the Psilophytales level."

He also makes the statement that "The higher Gymnosperms, represented in the lower Carboniferous by *Pitys* and its allies, may have passed through an earlier Pteridosperm stage, but this is not proven. The Spermatophytes generally, for all we know, may be as ancient as any vascular plants."

Early stages in the development of sporangia of Devonian and early Carboniferous Pteridophytes and seed plants are not favorable for preservation; but, even here, a section is secured now and then which indicates that Paleozoic sporangia followed the same lines of development which we see in living forms. A section of *Bothrodendron* from the upper Carboniferous of Illinois, recently described by Reed,[5] shows four megaspores and a plasmodium-like mass which can hardly be anything else than the broken down remains of wall cells, tapetal cells, and abortive spores. The resemblance to a sporangium of *Selaginella* at this stage, in which the origin of the mass is definitely known, is striking.

In all the living genera of heterosporous Pteridophytes, the homosporous ancestry is unmistakable. One needs only to recall the development of the megasporangia of *Selaginella* and *Azolla*.

In the megasporangia of some Gymnosperms, like *Pinus*, *Taxus*, and *Gnetum*, and even in those of some of the Angiosperms, like *Casuarina* and *Ranunculus*, there is considerable sporogenous tissue, from which more than one megaspore may begin to develop. The process of development is the same as in the heterosporous Pteridophytes.

We believe that it is in accordance with fundamental principles of development that vascular plants, in their phylogeny, pass through an homosporous

[5] Reed, Fredda Doris, Flora of an Illinois Coal Ball. Bot. Gaz. **81**: 460–469. 1926.

stage, and then through an heterosporous Pteridophyte stage, before they reach the seed stage. Consequently, we could not agree with Scott, that the seed plants may be as ancient as any vascular plants. While they were in the heterosporous condition, but not yet up to the seed stage, the predecessors of the seed plants were Pteridophytes, and this would be true, whether they were coming from some known Pteridophytes by direct descent, or were coming by parallel development from some Pteridophyte ancestry not yet discovered.

When structures like the leaves are so similar in ferns and seed ferns that the two groups cannot be distinguished by their leaves, it seems more probable that the sporangia—and also the vascular system—of the higher group have advanced from the heterosporous fern condition to the seed condition, while the leaves have remained stationary. It is logically possible that leaves so identical as to be indistinguishable may have developed in ferns and seed ferns from entirely unrelated ancestors; but it seems more likely that the similarity is due to genetical relationship. A Latin poet, in giving advice to young play writers, once advised them not to bring a god upon the stage unless the situation demanded it. In the case of ferns and seed ferns, it would seem that genetical relationship, functioning as it is known to function in living forms, where the relationship is fairly well known, is a sufficient factor to account for the observed structures without putting upon parallel development the strain of producing, in two great groups of plants, leaves so identical that they cannot be distinguished.

When Wieland goes to some new place we soon hear that there are Mesozoic fossils where they were not known to exist before. It is too early in the history of paleobotany to lay much stress upon the geological record, because extensive studies have not extended much beyond the University zone of the northern hemisphere.

The Rhynie fossils, as it seems to us, have no more bearing than other Pteridophytes upon the origin of seed plants. The sporangia of a form like *Hornea*, with its spores in tetrads, would suggest that the germinating spore would give rise to a gameophyte bearing eggs and sperms and that the new *Hornea* plant would come from a fertilized egg. The columella, which has led some to suggest a Bryophyte relationship, is no more highly developed than the columella—or subarchesporial pad—of some species of *Lycopodium*.

The ferns must have had an ancestry, but the Rhynie fossils seem to us to be already started on a lycopod, rather than a fern line of development. As far as their sporangia are concerned, they do not resemble either group.

For difficult and uncertain relationships, we need not confine our attention to fossils, with their incomplete records and almost entire lack of younger stages in development. Even in the living Angiosperms there is enough of difficulty and uncertainty.

Some lines of development seem well established: flowers with a spiral arrangement of parts preceded those with cyclic arrangment; hypogyny is more primitive than epigyny; the pentacyclic flower is lower in the scale than the tetracyclic; isocarpy preceded anisocarpy; and an embryo-sac developed from one megaspore is more primitive than a sac developed from two or more. An

hypogynous flower with a spiral arrangement is lower than an epigynous flower with the cyclic arrangement; but this does not mean, necessarily, that the higher flower has been derived from the lower, because the higher flower may simply have progressed farther than the lower along some fundamental line of development. Both may have developed independently from some plexus of plastic forms.

In accordance with these evolutionary tendencies in the flower, the Archichlamydeae have been arranged in a line from the Amentiferae to the Umbelliferae; the Sympetalae, in a line from the Ericaceae to the Compositae, and the Monocots in a line from Pandanales to the Orchids. Just now, these lines seem to represent an approximately true course of development; but this need not mean that each succeeding order must be derived from the one below it. We should be slow to believe that the cattails, aroids, and palms are the ancestors of the orchids, or that a huckleberry finally became a sunflower. But, for all that, we believe that there are tetracyclic flowers which have come by direct descent, from pentacyclic, and that much of the structural similarity may be due to genetical relationship. However, the possibility that similar structures may be due to parallel development and environment must be recognized. It may be that most of the forms in the three great lines of Angiosperms have come by parallel development from a few great centers like the Rose-Legume plexus in the Archichlamydeae.

In a great mass of forms, like the Tubiflorales in the Sympetalae, we should guess that there has been some parallel development, but also a great deal of genetic continuity.

To take an illustration in a group with which we are more familiar, in the cycad, *Macrozamia*, there is a group of species closely resembling *M. spiralis*. It would be hard to arrange all these species in linear order, each one derived from the one before it. More probably, some of these species have come, independently, from the highly variable, *M. spiralis*. However, if two of the species differed somewhat from *M. spiralis*, but closely resembled each other in the structure of the gland and venation of the leaf, it would seem much more probable that they were genetically related to each other than that both had come, by parallel development, from a common source.

In the Gymnosperms even the open carpel, from which the group is named, is not more universally present than the free nuclear period at the germination of the megaspore. The free nuclear period, at this stage, is more characteristic of living Gymnosperms than the single cotyledon is of Monocots, or the two cotyledons are of Dicots; but we should be slow to claim that the free nuclear period is due to inheritance. It is a natural consequence of heterospory. Homosporous forms do not show any free nuclear period and, as heterospory was just beginning, there was probably no free nuclear division. Some of the living heterosporous Pteridophytes have no free nuclear division at the germination of the megaspore; but with the heteropory in such an advanced stage as in *Selaginella* and *Isoetes*, the nuclear figure is too small to segment the comparatively large mass of cytoplasm, and a more or less prolonged free nuclear stage

followed as a necessary consequence, and became established. After a feature like this has become thoroughly established, we believe that it would continue even in small megaspores, as in *Taxus*, where one or two small megaspores often begin to develop, in addition to the larger megaspore which is to complete its development.

A free nuclear period in the embryo of Gymnosperms, immediately following fertilization, bears a striking resemblance to that which occurs at the germination of the megaspore, and it occurs for the same reason—the first nuclear figures are too small to segment the comparatively large mass of cytoplasm.

These two periods of free nuclear division occur in both the Cycadophyte and Coniferophyte phyla, which may not be even remotely related to each other. The extent of the free nuclear period, in both endosperm and embryo, in both phyla, depends upon the comparative size of the nuclei and the body to be segmented. In *Dioon edule*, with an egg 6 mm. in length, there are about 1000 free nuclei before segmentation begins; while in *Zamia floridana*, with a smaller egg, there are only 256 free nuclei.

In the lower, extinct members of the Cycadophyte line, the free nuclear stage has not yet been observed; but the endosperm is very small and, consequently, the eggs must have been still smaller, so that the free nuclear period was probably much shorter than in living forms.

In the living members of the Coniferophyte line the endosperm is small and the eggs are much smaller than in the living Cycadophytes, and it is well known that the free nuclear period in the embryo is very short—not more than 16 free nuclei in any of them, 8 in *Taxus*, 4 in *Pinus*, and none at all in *Sequoia* (which has a very small egg), a wall being formed at the first division of the fertilized egg.

In all these cases, free nuclear division occurs because the cell has become so large that the small nuclear figure cannot segment it. The free nuclear period in the early stages of the female gametophyte of *Pinus* is very similar to that of *Dioon*; the similarity, however, is not due to genetical relationship, but to similarity of conditions. Similar conditions have resulted in the production of similar structures.

A comparison of such structures shows how far a species has advanced along a certain line of development; so that one may determine, with more or less certainty, which species are more primitive and which ones are more advanced; but it may or may not be true that the one which has advanced the farthest is derived from the one which has not advanced so far.

In the Angiosperms the female gametophyte has become very small and the free nuclear period has become correspondingly reduced—not more than 16 nuclei in any form, usually only 8 and, occasionally, only 4.

We should not lay much stress upon the presence of a free nuclear period as an indication that the Angiosperms have come from the Gymnosperms. The free nuclear period is present because the megaspore, although small, is nevertheless too large for the first nuclear figures to segment it.

In the endosperm, following fertilization, there is likely to be no free nuclear division in small, narrow sacs; while in large, broad sacs, the free nuclear period is likely to be extensive.

The presence of free nuclear division in these various plants does not necessarily indicate genetic relationship. The egg of Angiosperms is very small, like that of Pteridophytes, and both begin to segment at the first division after fertilization; and they both segment not because they are genetically related, but because both have small eggs.

We desire to be fair, and we are willing to admit that morphologists have laid entirely too much stress upon phylogeny and have attributed to direct genetic development many structures which may be the result of parallel development due to fundamental principles of evolution. We even suspect that some of the phenomena usually attributed to recapitulation may belong here, so that a form may not be passing through a certain stage because that was the adult stage of some ancestor, but because that stage has a natural place in some fundamental sequence of evolution.

After making all these concessions, we believe that there are many similar structures in which the similarity is due to genetic relationship. Morphologists should recognize that there may be similarity, without genetic relationship; but, on the other hand, physiologists and ecologists should recognize that heredity is, and always has been, the dominant factor in evolution.

VASCULAR ANATOMY AND PALEOBOTANY[1]

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Latent in the minds of many, perhaps most botanists, is the idea that if the fossil record of plants were complete the problem of their genetic relationships would be solved. The idea is probably a subconscious heritage from the immediate past scientific generation (the Darwinian) whose view of evolution was that of plasticity of life forms, moulded by the hand of environment. However, with the present day shift of opinion, initiated by Weismann and fostered by modern geneticists, the conception of germplasm as a non-plastic, recalcitrant material has grown, and the adequacy of the fossil remains, however complete, to solve the genetic problem has come to be seriously questioned.

When, however, we turn to the geneticist for help we see that extremely complex and intricate as are the more minute relationships that he is slowly unravelling in the world of living plants, his method of attack is sufficiently difficult and restricted so far as the living is concerned but impossible with the dead. Futile too would be the effort to determine a mutation or germ variation among the fossil plants, when the determination in living material is so difficult and controversial. And yet, though the geneticist deals with a very limited field of relationships, shall we say the more superficial, such as distinguish species and varieties, that is, the ultimate branches of the phyletic tree, most valuable light is thrown on at least part of the problem from his results. Not only so, but other aspects of plant study have a contribution to make. In fact, the study of the living plant must ever provide the criteria by which to judge the phyletic value of the fossil. This has been realized to a considerable extent so far as morphology and anatomy are concerned, but to a markedly less degree in the case of ecology, physiology, plant chemistry, pathology, etc.

To illustrate something of the importance of one of these, ecology, brief reference will be made to some work[2] Dr. Sifton and I have recently done on resin tissue. We have shown that all resin canals, both horizontal and vertical, in the secondary wood and bast of the spruce are due to wounding. Previously, canals of the scattered type were regarded as normal features of spruce wood, while only those that were in tangential series were recognized to be traumatic. It was thus quite possible to have two detached portions of the same tree assigned to distinct genera on the basis of the presence or absence of resin canals. Such mistakes in the determination of fossil material would invalidate phyletic inter-

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany (Symposium: An evaluation of the structural evidences for genetic relationship), Ithaca, New York, Aug. 19, 1926.

[2] Resin canals in the Canadian Spruce, Trans. Royal Society, London. B, **214**: 63–111.

pretations. Again, false annual rings were shown to be produced on wounding, either accompanied by resin canals or not. Here there is still further liability to error. Wounded material too, because of the greater amount of resin preservative in it, is much more likely to become fossilized than normal material. As more is known of the influence of ecology on structure, greater need of guardedness in interpretation will be recognized and an increasing series of difficulties will become apparent to those who are endeavoring to trace the records of fossil plants.

Again, when the exigencies of fossilization are taken into account it is apparent that the fossil record can never be complete. More and more material is being and will be found, but the amount of petrified material with internal structure preserved is relatively small, and it is such material that is of most value, despite the wonderful work of the impressionist school. Here let me pay a tribute to Nathorst and the technique evolved and applied by him and his successors with such eminent and painstaking success. The petrified, sectionable material, however, must always prove more important. The influence of the discovery of such material in the past indicates the importance of the contribution that fossil botany is capable of making to the solution of the broader aspects of the problem of genetic relationship. I shall refer briefly to some of these outstanding discoveries of the past and their influence on our phyletic conceptions, and then make some suggestions with regard to certain recent discoveries in which we have become specially interested at Toronto.

Before doing so, however, I wish to draw your attention to the large number of most distinguished fossil botanists who have passed to the Great Beyond since the last meeting of the International Congress. Would it be your wish to stand while our Chairman reads this Roll of Honor. [Dr. Chodat then read the list, the audience standing]:

KIDSTON, LIGNIER, NATHORST, POTONIÉ, RENAULT, SOLMS-LAUBACH, ZEILLER

In the first place it is largely from fossil botany that we have learned of the significance of secondary growth. When this type of thickening was first described in certain ancient Cryptogams its discovery was taken by such eminent men as Brongniart and Renault as an indication that the plants concerned were dicotyledonous. Unger (1845) tried to controvert this view in the case of *Stigmaria*, which he held was not a Dicotyledon. Williamson, however, was the chief opponent. Of his service in this connection Scott,[3] who collaborated with him in so much of his work, says:

> "It was one of Williamson's greatest services to science that he always, in the face of much opposition, insisted on the true "exogenous growth" of the Calamites, while maintaining with equal decision their Cryptogamic nature. He thus established one of the most striking instances of homoplastic modification, for the close agreement in these anatomical characters between certain Cryptogams and Phanerograms is not, in itself, any proof of affinity."

We know now that secondary growth carries with it the potentialities of the tree-habit, a habit which was realized extensively in the spore plants of the past,

[3] Studies in Fossil Botany, Vol. 2, p. 410. 1923.

especially in the Lycopsid line (for example, *Lepidendron*, *Calamites*, etc.) where it is retained to a remarkably less extent at present; in the progenitors of the Conifers and in the living forms themselves; also in practically every order of the living Dicotyledons, with aberrant attempts in the Monocotyledons.

The discovery of Seed-Ferns a quarter of a century ago was preceded by some fundamental work on the anatomy of the Cycadofilices which clearly associated these forms with the Ferns and Cycads, as the name implies. This work prepared the way for the final, though none the less splendid discovery of the seed itself, which was delegated, however, to other workers. Anatomy too has had its proper recognition at the close, since Weiss has demonstrated that the root structure conforms with that in the seed plants by showing that the plane of the poles of the lateral root in *Lyginopteris* lies in the same plane as that of its parent. The result of the work on these Seed-Ferns has left us with but few true Ferns, when at one time we had a whole "Age" devoted to them, and has clarified our ideas as to the relative importance of the Lepto- and Eu-sporangiatae in the past.

The results of Wieland's remarkable discovery of the monoecious condition in the Cycadoidea illustrate a reversal of opinion similar to that in connection with secondary growth. At first these plants were thought to be pro-anthophytes but are now, I believe, pretty generally considered as a "dead end" of Cycad development.

In the work on the Sphenophyllales we see another field where fossil botany plays an important rôle, in the determination of synthetic groups. Fewer such, however, have been found in the past than at first was hoped. We see rather in the fossil record the long continuation of the main lines as we know them today. A statement of Dr. Scott's[4] in connection with the ancestry of seed plants is significant of the present attitude:

> "The present evidence indicates that the Spermophytes have been an independent phylum from very early times, and were not derived from Ferns or any of the higher Vascular Cryptogams, but more probably from some long-extinct stock, perhaps comparable to the Psilophytales,"

May we not, however, see a ray of hope in Dr. Hamshaw Thomas's (1925) recent discovery of the Caytoniales, Fern-Agiosperms as he calls them, and a possible need for readjustment of our ideas in respect to this matter.

The Rhynie beds of Scotland have recently provided most notable fossil material. Kidston and Lang's epoch-making discovery of the Rhyniaceae and the demonstration of their connection with the Asteroxyleae under the proposed name Psilophytales (in honor of Dawson's original work) were, however, almost coincident with a most important contribution on the living plants. I refer to the discovery of the gametophyte of the Psilotales by Lawson and Holloway (almost simultaneaously) and the careful description of the embryogeny and structure of the young sporophyte of *Tmesipteris* by the latter. These two discoveries are fundamental to the work that has been undertaken at Toronto.

This work was started because of the need of a basis in the lower vascular

[4] Op. cit. Vol. 2, 415.

plants for an interpretation of certain seed and seedling structures of the lower Gymnosperms that Miss E. R. L. Reed is investigating. It was found that although much and most valuable work had been done on the embryogeny and sporeling structure of the lower vascular plants, the results had not been correlated so as to be useful for comparison with the features of the seedlings being studied. In fact no distinction between the two main groups of the Pteridophytes—the Lycopsida or stem-dominant, and the Pteropsida or leaf-dominant—appears to have been recognized in the extensive literature on the subject, although the work of Jeffrey has placed this distinction beyond reasonable doubt in so far as the adult forms are concerned. The detailed results will appear in a joint publication ["On the Origin of Root, Stem and Leaf, and the Seed-Habit Features of the Vascular Cryptogams,"] of which my further remarks will be a synopsis.

Early in the course of the work it was found that the problem of the origin of the root was involved. With regard to this there is an anomalous situation. The root because of the centripetal structure of its wood (which is frequently referred to as Cryptogamic) is regarded as a very *primitive* organ, and yet in origin it has been commonly held ever since Hofmeister's time to be *adventitious.* This anomaly in viewpoint is considered due to laxity in the use of the term rhizome, the term being applied indiscriminately to underground (and even aerial) parts in the whole series of vascular plants, when strictly speaking these structures are of very diverse grades or organization. Had a distinction been kept to the fore we should have been prepared to recognize the more readily the root potentiality of the primitive type of "rhizome"[5] which is found in the lowest vascular plants, the Psilotal and Psilophytal forms.

Our view is that the proto-type of the Lycopsid "root" is to be seen in the young sporophyte or *Tmesipteris* which Holloway[6] states is "all rhizome." Here the first subterranean member ("root") arises not as an adventitious-member but concurrently with the subaerial ("shoot"), these first organs of the sporeling being formed by an equivalent dichotomy of a primary axis with the foot as its base, and being interchangeable in function. Even the cellular details of the dichotomy by which they are produced are illustrated in Holloway's detailed work and the common form and structure of these fir t two members ("root" and "shoot") emphasized. The mode of origin and the structural equivalence of "root" and "shoot" in the sporeling of other living Lycopsida (*Lycopodium*, *Selaginella*, *Isoetes*) conform with and confirm this interpretation. The similarity in the structure of the "rhizome" and the "shoot" in *Rhynia* puts the *adult* condition here into line with the *sporeling*. *Asteroxylon* shows a further differentiation not only in the "shoot" but in the "rhizome," the descending branch of which is more root-like than the horizontal.

In the lower Pteropsida (ferns) there is also evidence in the organization of the sporeling that the "root" does not arise adventitiously, but as a member

[5] In placing double and single inverted commas about the names of certain organs of the Lycopsida and Pteropsida respectively, we are extending a practice initiated by Dr. Scott (op. cit.).

[6] Trans. New Zealand Institute, **50**: 1918; **53**: 1921.

equivalent to the "shoot." There is, however, a marked advance in structure in the fern root, associated in the cases investigated with a combination of two leaves in its formation. In the fern the sporeling root is typically *diarch*, and of distinctly higher (seed plant) type than in the Lycopsida. This is considered a most important and fundamental distinction.

There is an equally important difference in the aerial members in these two groups—the difference between *microphylly* and *megaphylly*. Thus from the first stage the fern sporeling has much superior absorptive and assimilatory organs ("root" and "shoot") to those of the Lycopsid forms, which is reflected in the building up of the whole plant body. It is this that is considered to have been the basic vegetative feature in the success of the Pteropsid series which culminated in the seed plants.

The combination of superior absorptive and assimilatory organs is accompanied in the Eusporangiatae by a massive and hypodermal development of the sporangia, a correlative feature the importance of which has long been recognized. There is also in the Eusporangiatae prolonged endoprothallial nursing. This is enforced particularly in the Marattiaceae by the location of the archegonia on the lower surface of the large, thick prothallia. In these forms the absorption of food material by the whole embryo is prolonged until a potentially dicotyledonous embryo is developed, the function of the foot, the important nursing organ in the vascular cryptogams, which free their embryos early, being thus replaced.[7] In addition, in the Marattiaceae, the embryo is oriented so that its root apex is directed towards the neck of the archegonium. Both of these features are important characteristics of seed organization, more important, we consider, than the attainment of megaspory, which up to the present has received much more attention from botanists. In the Marattiaceae the plane of the lateral root may be parallel to that of the parent root, as in the seed plants.

In the case of such Lycopsid sporelings as have prolonged endoprothallial development similar to that of the Marattiaceae, for example, *Selaginella*, it is the "shoot" that is developed to a corresponding degree in these *stem*-dominant, or microphyllous forms.

Two further conclussions follow from the interpretations outlined above: (1) diarch root structure is primitive for the seed plants, and (2) the dicotyledonous condition has preceded the monocotyledonous in descent. Thus the comparison of the organization of vegetative and reproductive features in the Lycopsid and lower Pteropsid forms indicates the Marattiaceae as the family possessing the greatest combination of seed-habit characteristics among the Vascular Cryptogams. It also affords a basis for determining primitive features of seeding structure, such as diarchy of root and dicotyledony of seed.

[7] In this connection it may be stated that in our opinion the suspensor is an organ of occasion.

[8] Since this synopsis was presented the writer has put together evidence to show that megaspory is not essential to the seed habit (Trans. Roy. Soc. Canada, Section 5, 1927).

SOME FACTS OF MORPHOLOGICAL CONTINUITY AS SHOWN BY A COMPARISON OF FOSSIL AND LIVING PLANTS[1]

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I

I have been interested for many years in plant anatomy in relation to fossil structure. It is my belief that if there has been any continuity in plant evolution the same notation would apply to fossils as to living plants more or less related to these.

It is true that paleobotany has been unable, and will perhaps never be able, to trace to the starting point the origin of any of the living orders. But if we are to be able in the future to construct the genealogical tree of the vegetation of the past, paleontology will nevertheless have added greatly to our knowledge of the plant kingdom and will have given us a much fuller and juster appreciation of the relations between the different groups.

Now the paleontological discoveries of the last twenty years have, in my opinion, so profoundly modified the fundamental conceptions of shoot, axis, leaf, bundles, and sporophylls that in the near future we shall have, I am convinced, to reconstruct general morphology on new lines, or to adjust, at least, our former ideas to the new facts brought to light by patient and tedious paleontological researches.

There are, perhaps, botanists who will feel that I am here engaged in a very speculative enterprise, and they may agree with some of the best morphologists in underrating the value of paleontological facts in order to find not only common ancestors but essentially to fix better names of morphological and histological conceptions of fundamental value. The main objection brought forward is that the paleontological records are too discontinuous, too fragmentary, to constitute a secure basis for a reconstruction. The same objection is to be made to comparative morphology of the existing vegetation. The latter is also only a small part of the life-stock of the successive ages.

The first fundamental morphological conception to be discussed is the leaf. In dicotyledons this member, the so-called simple leaf, of the laurel type, is not a fundamental and primitive entity but a simplified plant organ, retaining from the complications of its past history the teeth on its margin; the latter being very likely the rudiments of former ramifications of a formerly branched organ. The ontogeny of the leaf points to that conclusion; for in the bud those ramifications

[1] Presented before the International Congress of Plant Sciences, Section of Morphology, Histology, and Paleobotany, Ithaca, New York, Aug. 19, 1926.

are as a rule more developed, and it is by the further development of the so-called phyllopodium that they are kept in the background, and sometimes almost to the extent of vanishing, through the preponderance of intercalary growth. Should this idea be accepted, this form of leaf would represent the morphological terminus of a long series of transformations.

Now the monocotyledonous leaves, with a few exceptions, do not correspond to this type; they show neither in the developing bud nor in the adult condition the so-called ramifications. But I will abstain here from dealing with this special problem (compare the work of Arber, in several publications). I will, in fact, only point out that while very often in amphibious dicotyledons the simpler terrestrial leaf-blade is, by submersion, replaced by the well known highly dissected leaves, such as are exhibited by certain forms, notably *Ranunculus*, *Hottonia*, *Bidens*, *Limnophila*, and others, these leaves seem to constitute an atavistic reversion.

Now in a recent paper Bower insisted, perhaps more than any other morphologist before him, upon the point that in pteridophyta and especially in ferns the leaf of the primitive forms is highly divided, the ramifications arising, as a rule, as the consequence of a dichotomous branching, this latter passing, very often by the more rapid evolution of one of the two primitively equal parts, to a sympodium. When the leaf shows an entire blade, or a less diversified lamina, this is attributed to a so-called coalescence of the ramifications. In a similar way the simple laurel leaf of the dicotyledon is, as stated before, a simplification. Now this theory will apply in the same way to the leaves of almost every group of the pteridophyta, the most ancient representative having possessed a more highly subdivided lamina than the types of the same order of later origin.

The foregoing principles, stated in a necessarily brief manner are, in ferns at least, based not only on the evolution of the leaf, starting from the sporeling and up to the adult stage, but they also hold good if we consider the phylogenetical series as recorded by paleontological researches.

II

It is also a well known fact that by its apical growth the fern leaf occupies a peculiar position in the general morphology of vascular plants, showing something of the nature of a shoot, and for this reason having received the name frond.

All the recent discoveries made by phytopaleontologists reinforce, therefore, a theory first proposed by Potonié (1877) but more completely exposed by Lignier (1903). Lignier made a distinction between two kinds of foliar appendages: hence the "cauloids" of the ferns and of the phaneroganus and the "phylloids" of the Lycopsida. But Lignier's cauloid theory implies also the fact, or presumption, that the most primitive of these fronds were quite deprived of foliaceous flattenings. He pretended that, later on, by a process called by him "cladodification" these forked or branched cauloids became progressively flattened and were more or less provided with leaflets or "pinnules."

At the time when this theory was formulated by Lignier (also partly by Potonié) it was founded on very little paleontological evidence. This French

botanist nevertheless connected his experiences on the morphology of the living pteridophyta with the conclusions drawn from the knowledge of the very curious type of pteridophyta belonging to the Lower and Middle Devonian strata of Canada (Gaspé), the now well known *Psilophyton princeps* of Dawson (1859).

At about the same time Potonié and Bernard (1904) described from the Middle Devonian of Bohemia (étage H de Barrande) a certain number of Pteridophyta all or almost all represented by dichotomous, naked cauloids deprived of any foliaceous appendages, but presenting at the apex of the shoots (cauloids), very often, the characteristic curvature of the fern fronds. They at once perceived that those plants corresponded to pteridophyta, allied to the ferns. They discovered the vascular structure in some of them (*Hostimella*, *Pseudosporochnus*, *Spiropteris*, and others.

We may also recall Tansley's statement, in his lectures on the evolution of the filicean vascular system (New Phytologist, 1908) that the fern leaf is in phylogenetic origin a branched system, not an appendicular organ differing *ab initio* from the axis on which it is borne.

But it was only after the publication of the important paper by Kidston and Lang on the Rhynie fossils, with structure preserved, that the attention of nonpaleontologists was directed to former contributions on the same subject. The leafless cauloids of the two principal genera of this age, *Rhynia* and *Hornea*, were dichotomously branched and sprang from a rootless rhizome and were provided with large terminal sporangia. The anatomical study revealed in the axis a simple stele.

But previously, Halle (1916) had published a very good account of the fossils of the Middle Devonian of Röragen in Norway, in which he describes many interesting fossils of the same type. These also were deprived of any marked pinnules, and his conclusion may be quoted here: "The most obvious negative characteristic of the lower Röragen flora, and, according to Dawson, also of the Gaspé flora, is indeed the absence of any fern pinnules or other foliar laminae."

A very fascinating account of the Devonian floras was given out in 1921 by the late Newell Arber. Unfortunately his conclusions that *Psilophyton* Dawson, *Hostimella* Stur, and also probably *Rhynia* belonged not to the vascular cryptogams or to pteridophyta seem not to be correct and, so far as I know, have not been admitted by competent paleobotanists. Later on Scott, Nathorst (1914–1915), Seward, Weiss, Pia, Kräusel, and the author of this review have given their opinion as to the bearing of those discoveries. All those agreeing with Lignier have compared the leafless fossils of the Lower and Middle Devonian (this flora presenting the same aspect in the various deposits of the world; compare Stolley 1925, Seward 1923, Arber 1921) with the well known *Psilophyton princeps* of Dawson. This fossil was therefore considered as the type of a new class of plants. Furthermore, Kidston and Lang (1923) published a very striking photograph of a new representative of that order, *Hicklingia Edwardii*, so well preserved that this impression reminds us of the old idea of Scheuchzer when he calls the slates on which the plant remains are printed "Herbarium diluviale." Lang a year ago 1925, attributed a new fossil of the old red sandstone of Holland

to the genus *Hostimella*, *H. racemosa*, apparently leafless but bearing a simple raceme of reproductive organs in which I should be inclined to see seed-like organs rather than sporangia of the type of *Hostimella*. This plant is accompanied in the same locality by a great variety of spores, some of them suggesting Lycopodian affinity. These spores are remarkably large for spores of homosporous pteridophyta or small for macrospores, whether of pteridophyta or pteridosperms. This would indicate that, at this level, the two great groups of pteridophyta were already differentiated or in process of differentiation, and these results would be in accordance with the former observations of Potonié and Bernard on the Étage H de Barrande.

According to Lang, *Thursophyton Milleri* Nath., a dichotomously branched system of the same origin, which was covered with phylloids as in a lycopod, exhibited also emergences, much in the same way as *Protolepidodendron Scharyanum*, presented at the apex of the ramifications the circinate, coiled condition of a fern frond.

Lastly, we may quote here an important paper by Kräusel and Weyland of the Devonian flora of Elberfeld, which has just appeared, and in which they show, extending the prophetic results of Nathorst, Halle, Kidston and Lang, that there existed a plant combining on a single shoot the morphological and anatomical features of three Devonian fossils considered till then as distinct species: *Psilophyton princeps* (of Dawson), *Hostimella hostimensis* Stur (after Potonié and Bernard) and *Thursophyton Milleri* (Sael.) Nath. They also pointed out that the latter correspond very nearly to the now well known *Asteroxylon Mackiei* Kidston and Lang, one of the Rhynie fossils. It is, of course, always rash to pretend that a given fossil from one station is identical with another species of a remote locality. But here the new fossil combines really in itself the different characters found in those different plants. The rhizome recalls that of *Psilophyton;* it is deprived of root, bearing rhizophores of a sort much like those of a *Selaginella;* the erect cauloids are in their inferior parts covered with small, scale-like projections, very similar to those of *Asteroxylon;* these pass higher up to spinous projections, such as have been described for *Psilophyton*, the ultimate ramifications of this fossil, which attained a length of several decimeters, were naked and correspond exactly to the so-called *Hostimella hostimensis* Stur. Now the upper naked part exhibits a single stele provided (but this point is not quite certain) with a central protoxylem; lower down the primary wood, in section, shows the stellar arrangement of the xylem of *Asteroxylon Mackiei* as described for this fossil of the old red sandstone of Scotland by Kidston and Lang. This fossil combines, accordingly, in one specimen the characteristics given by Lignier for the two classes of pteridophyta, naked dichotomously branched cauloids with coiled apices, and also cauloids with small appendages, recalling the leaves of *Lycopsida*.

The same idea has been formulated by Scott in his Studies (III, p. 413, 1920): "The supposed fertile axes of the Devonian plants were of the nature of leaves, or at least were on the way to become leaves. *Asteroxylon* would have possessed two kinds of leaves, or incipient leaves—the fertile axes, no doubt modified branches, and the small leaves clothing the stem; the latter would be

comparable to the phylloid of Lignier, the characteristic leaves of the *Lycopodium* phylum. Lignier regarded his phylloids as leaf-like emergences and as quite distinct in origin from the true leaves of the megaphyllous line."

But it would take me too far away from my subject to discuss here the important question whether "the whole pteridophytic stock would be monophyletic, the *Lycopsida* and the *Pteridopsida* being derived from a common form already vascular.' The restoration by Kräusel and Weyland of the *Asteroxylon elberfeldense* could be interpreted as an argument in favor of this theory.

But we know, however, too little of the Devonian flora to take such an attitude. Moreover, it is certain, from the discoveries of Kidston and Lang, and also of Kräusel and Weyland, that at the same period there were already stems with secondary thickening of the wood; *Palaeopitys Milleri* and the much older *Archaeoxylon Krasseri*, the latter of the Precambrian of Bohemia, perhaps the oldest wood structure known.

This and the presence of a variety of large spores of different kinds will suffice to prevent us from considering the Psilophytales as having constituted in Devonian times the only primitive rootstock for later evolution. Also the curious fern-like bodies, borne on the axes of *Hostimella racemosa* Lang point also to a pteridosperm affinity.

But up to the present not a single species has been found in the Lower Devonian beds provided with foliage-like expansions. The so-called pinnules of the slightly flattened cauloids, as they have been described from Lower and Middle Devonian strata, as, for instance, in *Hostimella pinnata*, were only somewhat flattened, though this hardly amounts to the differentiation of a definite lamina (Lang). Even in the Proto-articulatae, described recently by Kräusel and Weyland (1926), the degree of cladoidification exhibited by dichotomous lateral cladoids did not exceed the dilatations exhibited later on, in the Upper Devonian and in the Culm by the different species of *Sphenopteris* (*Rhodea* spec. of old), the supposed foliage of some pteridosperms.

Of course, in the upper Devonian we meet with a fair number of pteridophyta related, or belonging, to the fern stock, or perhaps to the Ginkgoales, provided with larger flattened pinnules, as, for instance, in some of the then very common species of *Archaeopteris*, *Cyclostigma*, or *Psygmophyllum*, whose dichotomous branchings were distinctly exhibited. But for our demonstration it is immaterial whether some of these forms had already passed the limit between spore plant and seed plants, for we are dealing only with the morphology and first origin of the vegetative system of the whole fern stock.

If we accept the views just expounded, the existence during the carboniferous age of a whole group of specialised fern plants of unknown relationship with any other order of ferns, which were characterized by slender, elongated, more or less dichotomously branched fronds sometimes disposed, apparently, along the main axis in four rows, will appear to us less surprising. Both Botryopteridaceae and Zygopteridaceae are, it is true, only known as fossil structures, and their actual appearance can only be guessed by reconstruction, through the aid of sections.

But we know, at least in *Stauropteris*, that the linear fronds were fern-like, coiled at the apex.

Whether in some cases the flattenings of the secondary branches of the bush-like fronds amounted really to a sort of pinnule is not known, except in the doubtful case of *Botryopteris*. Here too the paleomorphologists find some difficulty in calling these organs leaves, fronds, or the like. In some cases we know (*Anthyopteris*) that these were borne on rhizomes, or axes with a pronounced radial structure. But in the primary cauloids of the different species of this order the structure was not always a dorsiventral one, and in many of them the bilateral structure appeared distinctly only on the branches of the second order.

Now the same remarks apply to the fronds of *Lyginopteris* (*Sphenopteris* foliage). When the leaf trace started from the periphery of the medulla it retained, at first, the structure of the stem stele and only during its passage through the cortex it flattened and divided so as to adapt itself to the dorsiventral morphology of the highly ramified frond. But even here the mode of ramification of the main axis of the frond recalled, as I observed in well preserved fronds of *Sphenopteris* (*Rhodea*) *elegantiforme* from the Culm of Moravia, the mode of branching met with in *Stauropteris*, that is, it was in four rows. In the majority of the species of *Sphenopteris* the pinnules, curiously enough for such a large frond, remained so small that in many cases they can only be easily observed with the aid of a lens.

Now the morphology of the fern frond is too well known to require taking more of your time on this subject. But it will not be useless perhaps to add, as Lignier, Tison, and Matte have shown, that in the leaves of Cycadales and Coniferales, when there are more than one vein, the venation can always be explained by dichotomous branching. In the dicotyledons this mode of branching of the leaf has not completely disappeared, for instance in cotyledons (Bugnon 1922, 1923); but here the sympodial branching has become prevalent.

III

Passing on from the morphology of cauloids and fronds, we come to the sphere of histological problems, connected with the study of the same fossils. Without entering into details, I wish first of all to outline some of the leading principles of anatomical research which seem to me well adapted to establish the continuity of evolution in the conducting system both of fossil and living plants. The problem may be approached by neglecting the phloem system, the latter being seldom preserved in fossils, and besides that sieve tubes lack very often a definite correlation with the topographical disposition of the xylem elements. To establish the comparison I am inclined to believe that first of all the actual place of the protoxylem should be carefully ascertained. Whereas, in some fossil shoots the positions of this protoxylem can not be determined with certainty, its probable location may be deduced from its situation in the departing foliar bundle.

Secondly, it is necessary to know exactly the direction taken by the piling up, that is, the succession of the metaxylem from this very starting point, without

giving importance to the quantity of the so-called metaxylem elements (primary centrifugal or centripetal wood of the bundle). This kind of bundle development I called the "divergent," using this notation in several papers and pamphlets and in a textbook.

I proposed to apply this point of view to the reading of the topographical constitution of the primary conductive system. The idea was taken from that beautiful work of Bertrand and Comaitte on the anatomy of fern leaves. It is to be regretted that this contribution to the anatomy of plants is so little known, not only in England but everywhere. It seemed to me, and the new paleontological records contributed only to strengthen my position, that many difficulties of comparison should disappear using this mode of representation. Of course, every notation is only a mode of comparison of supposed homologous structures, and I am prepared to accept any mode of description which should, by its application, bring better results. The stelar theory ought to be abandoned, or put aside, because it does not take into account the two principles to which I just referred. Yet as a short descriptive method, to give an idea of the concentration or the disposition of the primary wood elements, as a whole, it can be employed with benefit in regard to physiological anatomy.

Now when we consider the disposition of the primary wood in the Psilophytales (*Rhynia* and *Hornea*), we find here a central strand of metaxylem occupied in its middle by smaller vessels—by the so-called protoxylem. But here the difference between protoxylem and metaxylem is not in the qualitative difference of the conducting cells, but only in their relative size. This is a very primitive and exceptional disposition of the primary wood.

Now in *Asteroxylon* this so-called protostele exhibits a stellate section, the protoxylem elements are situated very near the periphery and according to the divergent theory, every projection is considered to be built up of a group of protoxylem elements followed first in centrifugal direction by a small amount of metaxylem; then this latter expands horizontally, diverging by the successive addition of new elements, so as to form two lateral wings. (This portion constitutes what in the English paleobotanical literature they call centrifugal wood.) Later on the new elements of metaxylem are added in such a way that the wings seem to bend down and therefore bend to form a complete ring enclosing the protoxylem elements. This latter part of the primary wood (metaxylem) is called there centripetal wood.

Now very often the different bundles unite by the inner side of the so-called mesarch bundles by a super-addition of a central, medullary portion of metaxylem elements built up by continuous conducting vessels of a larger diameter than those of the first metaxylem of the ring-bundles. It is easy to make out that the protoxylem elements lie in direct connection with the small centrifugal part of the metaxylem, and for that reason I call this kind of bundle endarch. All this is derived by a comparison of these bundles in fossil plants with the analogous structures found in leaves of true ferns.

The condition encountered in *Asteroxylon* is also to be found in the stems, or so-called stems of Zygopteridean affinity, as for example in *Ankyopteris corrugata*, *A. Grayi*, and *Asterodilaena*.

Very often the protostele is not ribbed and the protoxylem elements lie disposed in a submarginal circle, near to the periphery of the primary wood. (*Stenomyelon*, *Palaeopitys*, pteridosperms). In more advanced types (that is we consider them more advanced because they seem to tend in the direction of the organization of plants of a later origin) the continuous, large-celled metaxylem is interrupted by parenchymatous cells, and this medullary wood becomes discontinuous, so, for instance, in the well known primary wood of *Heterangium* (*Sphenopteris elegans*).

The order Gleicheniaceae offers (Chodat 1917) in different species a similar modification from one type (protostele) to the other (solenostele), but here the solid protostele of some species becomes ring-shaped by the formation of a medulla; the situation of the protoxylem imbedded in the metaxylem of this solenostele is the same as in the preceding instances.

We may remember that in a type belonging to earlier Osmundaceae there is also a continuous ring of metaxylem, with the same localization of the protoxylem elements, while in certain representatives of *Todea* this ring is more or less broken, the protoxylem remaining in the same situation, this being particularly well observed at the very moment when the leaf trace leaves the stem.

Now *Lyginodendron* shows a still more advanced stage of evolution towards higher organization. Here the so-called monarch bundles, which I call "divergent ring, with endarch protoxylem" are individualized, forming at the periphery of a medulla a crown of peculiar bundles which have been often compared (it was an error) with an analogous structure found in cycads. The leaf trace of *Lyginopteris* maintains during its passage through the cortex an identical structure; later it divides forming two protoxylems.

The well known stem structure of *Osmunda* is another instance of the same kind, but here the ring is generally opened and its inner side exhibits a horseshoe-shaped section, with the protoxylem located at the inner angle of the Ω.

In Psilotaceae the bundles of *Tmesipteris* belong to the same type of structure; they are also disposed in a circle around a more or less vascularized medulla. Lang showed long ago that this condition is also to be found in some Ophioglossaceae, especially in *Helminthostachys*. It would be an easy matter to describe the various woody structures of the Zygopteridean cauloids, using the same notation, but here the two arms or wings of the "divergent" bundle are sometimes unequal, the closed divergents and the endarch situation of the protoxylem being particularly obvious in the outgoing pieces.

In the leptosporangiate ferns, excepting the Gleicheniaceae, Schizeaceae, Hymenophyllaceae, etc., another stem structure prevails, but it would take too long to discuss this. I shall only insist upon the fact that between the oldest fern-like plants, from the Psilophytales through the pteridosperms belonging to the *Lyginopteris* stock (*Calamopitys*, *Stenomyelon*, *Protopitys*, and others), also through the true ferns which always have been considered as primitive in structure, or which actually occur in very old times, a vascular structure prevails which is quite unknown either in cycads, Cordaitales, Ginkoales, or angiosperms.

In this I am sorry to disagree with the opinion of such a great authority upon fossils as Dr. D. H. Scott. I know he will forgive me, for it is long that we have held contrary views on this definite point, yet we have remained good friends. But today as in 1900 I can not see in the anatomical structure of the Lyginopterideae any point of likeness with the cycads. I would not call the former phanerogams merely because some of them, or perhaps many of them (we do not know), bear seeds or something like seeds. Also I would not be inclined to extract *Lepidocarpon* from the Lycopsida for the reason that we have here a seed plant.

Every detail of the stem and leaf morphology and of the anatomy points in the direction of the fern stock taken as a whole. The Lyginopterideae may have shown themselves at all times perfectly distinct from other ferns, as in the case of the Zygopterideae, Osmundaceae, and other orders, but they belong nevertheless, so far as I am able to have an opinion in the matter, to the seed ferns and not to the phanerogams. Now this question arises: Is there no connection between the successive structures just mentioned and in which we find an appreciable degree of morphological continuity (expressed by gradual changes, showing perhaps a better adaptation to new conditions) and the stem and leaf structures prevailing in conifers and angiosperms? Here the divergent bundle is open and endarch, when it closes up, as it sometimes does, the two arms of the bundle never bend backward but unite around the phloem, the protoxylem remaining outside. This latter behavior is also often found in monocotyledons.

But this morphological transition took place in a group of plants belonging to the pteridosperms. I refer to the genus *Calamopitys* (*Arthrodendron*), so fully worked out by Dr. Scott. Here a transition is found between the closed divergent bundle of *Calamopitys americana* and the open divergent, the latter better adapted to start with secondary thickening than the other. But we must await further discoveries before we can trace a continuous line of morphological continuity between the closed divergent and open bundles of the phanerogams.

The third point, connected with the two others just mentioned, on which I shall make some remarks in the hope of showing that we shall have to revise our ideas in respect to the matter of placentation. We are accustomed to think that sporangia (archidies) are borne on leaves, on the so-called sporophylls, and that when we can observe a distinction between a fertile and a sterile part of such a sporophyll the first-mentioned is a direct modification of the second. But here too paleontology clearly shows that tetraspores were present in primitive pteridophyta long before the above-mentioned cauloids had developed into flattened leaf-like laminae. The large sporangia of *Rhynia*, *Hornea*, *Psilophyton*, *Hostimella*, and of all known Psilophytales were borne on terminal and naked branches of the cauloids. This holds true with that old order of ferns, the Zygopteridaceae; and, so far as we are definitely informed, both microarchidia and megarchidia of *Lyginopteris* are similarly disposed, although the fronds of members of this genus, as well as of the family Zygopteridaceae, possessed fronds with leaf-like flattened pinnules, these being terminal and supported by naked stalks (cf. Carpentier, 1914).

In the Psilotales the sporangium is clearly terminal on forked cauloids. Here, too, the "sporangia" are usually large, often measuring 6–8 mms., as in *Tmesipteris*.

Now in the whole group of tree ferns we see that the sporangia, whether grouped as usual in sori, or isolated, are not directly inserted on the lamina but on a placentum (stalk) or sporangiophore (Bower). The sporangiophores take their origin from the veins, and these—without going too far in speculation—we may be permitted to consider as the vestigia of the dichotomous branches of the fern cauloids. From various workers on fern morphology, we know that the primitive condition seems to have been the marginal location of the sporangiophores or placentae. Progressively this marginal arrangement was modified, the sori becoming located more and more on the dorsal surface of the frond. But here too, with a few exceptions, they are borne on the veins. If, as generally claimed, the placentae on the archidia arising from veins are to be considered modified pinnules or leaflets, this removal would be a very striking one.

This theory of apical placentation on naked branch-systems of cauloids, which everywhere among vascular plants opens a new field for morphological investigation, is intimately associated with the interpretation of the morphological dignity of the socalled sporophylls, the stamens and carpels of the angiosperms.

A chief object of this paper is to direct the attention of morphologists and paleontologists to the very striking parallelism of successive modifications as observed from Psilophytales to the living ferns showing a primitive stem structure.

During the differentiation of the leaves from cauloids, as previously indicated, the anatomical structure maintained its unity, the apparently striking dissimilarities being of secondary importance (adaptations, etc.). Further, with regard to placentation, we see that the terminal sporangia of the Psilophytales and Psilotales are borne on cauloids becoming marginal in the case of many ferns of primitive origin—well illustrated by *Osmunda*—tending to pass to the dorsal part of the frond and retaining from their primitive terminal location their organization in sori, these last arising from the veins as specialized ramifications.

But I feel that I have already spoken too long. I only hope I have made it clear how, in my opinion, paleontology is to be combined with morphology and anatomy to solve, so far as possible, the difficult problem of the origin of the various structures of the higher plants.

SOME PROBLEMS OF PLANT ECOLOGY[1]

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The last decades have greatly increased our knowledge of the relations of vegetation to the conditions of the outer world. Much work in the way of experiments and observation has been devoted to the analysis of individual factors, which always act in a very complex manner and therefore are very difficult to analyze. The main factors that lend themselves to observation are climatic, edaphic, orographic, biotic, anthropic, and genetic, though of course this classification refers only to the origin of the factors and not to their real nature, as is correctly emphasized by H. Gams, who distinguishes only physical and chemical factors. From a practical point of view, of course, the classification given above is justified, even though today the correlation of the climatic and edaphic factors, for instance, is generally acknowledged, which fact is expressed in the classification of soils on a climatic basis, because certain types of climate (for example, arid or humid) create certain types of soil. Of course, there are also soils whose structure and properties are manifestly affected by the internal conditions of the evolution of the substratum; Glinka designates them as endodynamomorphic, in contrast to ektodynamomorphic soils, which are the result of climatic influences.

A detailed study of habitats and vegetation reveals everywhere in central Europe a continuous mosaic of ecological conditions and a corresponding mosaic in the plant covering. The comprehension of this fact is of paramount importance, because only in this way are numerous seeming discrepancies in the distribution of species and associations to be explained, and harmony brought into the relations of the vegetation to the habitat. The conceptions microhabitat and microclimate are therefore the basis of the study of ecology, but these, nevertheless, must not lead to the breaking up of plant communities into an endless number of associations; the conception of association must remain broad, and only within its limits should be carried out the subtle differentiation into types, facies, and variants, just as the conception of species (in the sense of Linnaeus) remains the basis for taxonomy. Strictly speaking, even the microhabitat is a complex of different habitats, and this we can observe best in associations with more than one layer, especially forests. Different species send their roots to different depths, and therefore into strata with different water balances, and usually also of essentially different chemical properties. Woodhead gives as an example of such a case an English oak woodland in which the rhizospheres

[1] Presented before the International Congress of Plant Science, Section of Ecology, Ithaca, New York, Aug. 20, 1926.

of individual species are each in a different stratum. *Holcus mollis* occupies the surface stratum, *Pteridium aquilinum* the stratum immediately beneath it, the bulbs of *Scilla nonscripta* a deeper stratum, and the roots of the oak a still deeper stratum. Strictly speaking, then, the general habitat is divided vertically into partial habitats, which brings to mind Gams' biotypes. Also, from the ground upward the habitat is divided into vertical zones, often essentially different from one another. A similar subdivision of the general habitat could be made also in other regards, and indeed the plants themselves do it in some cases, as we see in forests where the bark of trees forms such a habitat, which is itself differentiated according to its character, exposure, height above the ground, and so on. Nevertheless, it is to be recommended that the conception of habitat be preserved in its wider sense, for it can then be subdivided according to local conditions.

In this lecture I mean to discuss some regions of the Czechoslovak Republic, so that I may define with concrete examples the subtle relations of the vegetation to the mosaic of habitat conditions.

I. The Brdy Mountains and the pH Factor

The "Brdy" is an inland Bohemian mountain range, covered with a continuous forest and reaching their greatest height in the peak Tok (854 m.). It is a typical hercynian range, as I showed in my monograph in 1903, but it was overlooked by Drude in his work on the hercynian region, just as was the southern portion of Bohemia, the greater part of which has associations that are classical examples of the hercynian type. The position occupied by the Brdy in the whole flora of Bohemia may be seen from the regional division that I carried out in the year 1924. In the forest associations, spruce and pine (*Pinus silvestris*) forests predominate today, the mixed and beech forests having been decimated by forest culture. Today the soils of the Brdy are considerably acid, as I found through the analyses of a large number of samples. As an example, I give in Table 1 some especially characteristic types.

The individual forest associations have a relatively constant associational acidity. As examples I give below pH indications for pine forests with undergrowth of *Vaccinium*, *Calluna*, etc., in the woods around Příbram:

	pH
1. *Pinus silvestris*	4.5
2. *Vaccinium myrtillus*	4.6
3. *Luzula nemorosa*	4.5
4. *Vaccinium vitis idaea*	4.5
5. *Deschampsia flexuosa*	4.8
6. *Calluna vulgaris*	4.5
7. *Dicranum scoparium*	4.2

In this association, therefore, the factor pH oscillates between the extremes 4.2–4.8, and the average is 4.51. Another pine forest of the Brdy, representing the same forest association, shows an oscillation in the soil acidity of the rhizo-

spheres of its characteristic species within the extremes pH 3.8–4.9, the average associational acidity therefore being 4.4.

On a range shooting out from the Brdy, called Hřebeny, I analyzed a pine forest on the slope of Hřebeny above Řevnice, and described in detail its socio-

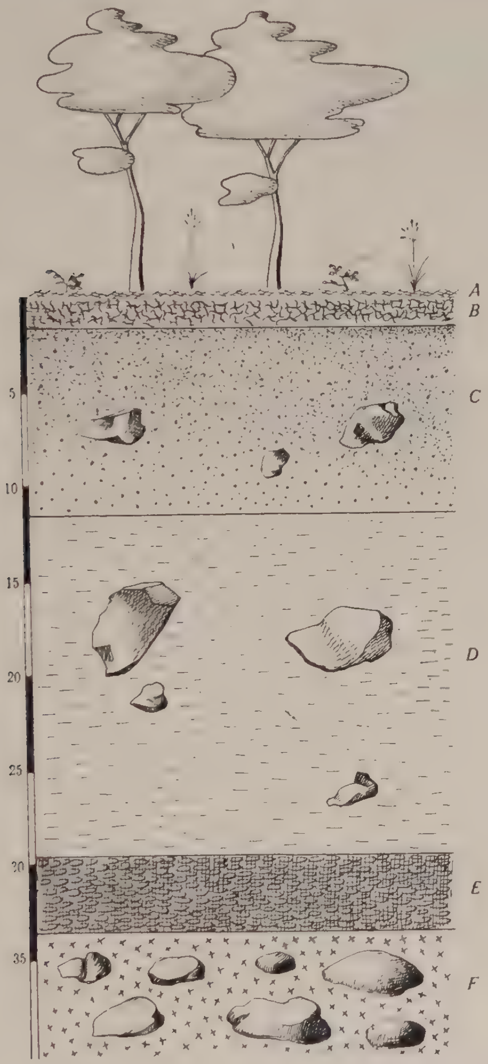


Fig. 1. Profile of forest soil on Mount Kŏcha in the Brdy Mountains: Pine forest with a monotonous hercymian undergrowth (mainly *Vaccinium vitis idaea* and *Deschampsia flexuosa*; A = half-decomposed pine needles, B = coarse raw humus, C = slightly infiltrated and leached out subsoil, D = leached out lower stratum, E = ortstein stratum, F = loam with stones.

TABLE 1. THE REACTION OF CERTAIN SOIL TYPES, WITH CHARACTERISTIC SPECIES.

Number of samples	Species	pH extremes	Average
7	*Calluna vulgaris*	3.8–5.5	4.83
6	*Deschampsia flexuosa*	4.4–5.4	4.88
4	*Dicranums coparium*	3.7–4.2	3.95
3	*Fagus silvatica*	4.9–5.2	5.03
3	*Festuca ovina*	4.6–6.2	5.40
4	*Luzula nemorosa*	4.5–5.3	4.85
4	*Nardus stricta*	4.0–5.2	4.55
5	*Picea excelsa* (humus)	4.2–5.2	4.72
4	*Vaccinium myrtillus*	3.4–4.6	4.01
5	*Vaccinium vitis idaea*	3.7–5.6	5.52

logical conditions in my work on the ecology and genesis of forest associations. The soil acidity of the characteristic individual species is here as follows[2]:

1. *Pinus silvestris*.................. 4.3 (4.0)
2. *Vaccinium myrtillus*.............. 4.6 (4.6)
3. *Luzula nemorosa*.................. 4.7 (5.4)
4. *Deschampsia flexuosa*............. 4.0 (4.3)
5. *Dianthus silvaticus*.............. 4.6
6. *Antennaria dioica*................ 5.0 (5.1)
7. *Anemone nemorosa*................. 4.5
8. *Calluna vulgaris*................. 4.2
9. *Leucobryum glaucum*............... 4.3 (4.3)
10. *Hypnum Schreberi*................ 4.5
11. *Polytrichum commune*............. 4.2

The soil acidity therefore oscillates between the extremes pH 4.0-5.0, the average being 4.45, which means that on the whole the associational acidity agrees with that of the growths given above. This fact was confirmed also by other analyses, as for instance that of the pine forest above Drahlín, which in the rhizospheres of its not numerous characteristic species showed the following acidity:

pH

1. *Calluna vulgaris*.................. 4.9
2. *Vaccinium vitis idaea*............. 3.8
3. *Vaccinium myrtillus*............... 4.5

The oscillation is greater here, but the average associational acidity is again pH 4.4.

In a certain sense acidity may be an indicator of the degree of naturalness of this or that forest association, as can be demonstrated in the case of the Pineta silvestris of the Brdy Mountains, which are divided into 3 categories, the sociological and ecological conditions of which I described elsewhere. These are:

A. Typical xerophytic forests of *Pinus silvestris* of the Brdy.
B. Forests of dwarfed pines on the ridges of the Brdy.

[2] The acidity of the mineral layer beneath the humus is given in parentheses.

C. Secondary forests of high-trunked pines, occupying the place of former mixed forests.

From this it is evident that associational acidity is here characteristic for the different types of pine forests, and indicates the degree of naturalness and the type.

The humus in the whole of the Brdy range is raw (decidedly acid) and for the most part coarse. Only in a few mixed forests is it somewhat different, of finer texture, and often less acid, but never real mould. Beneath the fir (*Abies alba*) as a rule there is richer, finer humus, which, however, is considerably acid (pH 4.6). The different types of forest humus lead me to a somewhat different classification, which may be summed up as follows:

A. *Acid humus* (raw humus in the widest sense of the word):
  1. Sphagnum peat.
  2. All other types of acid humus:
    1. Coarse humus; that is, raw humus formed of leaves, twigs, etc, that are as yet but slightly decomposed.
    2. Raw humus (true): more or less coherent and compact decomposed parts of plants together with an abundance of roots, underground stems, the mycelia of fungi, etc.
    3. Fine acid humus, thoroughly weathered and of the finest texture.

B. *Neutral to alkaline humus*:
  1. Coarse mould, incompletely decomposed.
  2. Mould (true), thoroughly decomposed, of finest exture.
  3. Artificial types of neutral to alkaline humus, as field soil, and agricultural, garden and all fertilized soil in general.

Fine acid humus behaves in its influence on the vegetation otherwise than coarse humus; up till now it is of course an unsolved problem whether or not the differing amounts of nitrogen present are responsible for this.

The layer of mycorrhiza, which Müller and Peklo ascertained in the case of the beech, is present also with *Acer pseudoplatanus* and is of importance for the process of humification and nitrification.

The evolution of the soil conditions in the Brdy can be reconstructed in its general outlines as in Table 2.

From this table, based on a detailed investigation of today's ecological and sociological conditions in the Brdy, it is evident that in the course of time very radical changes in the soil conditions have taken place, mainly in consequence of anthropic influences, which were supplemented by the humid climate.

An excellent aid for the reconstruction of the original conditions of vegetation is to be found in the relict localities and relict plant associations, which often are preserved only fragmentally. I devoted a great deal of attention to their study, and the results obtained through this new method are in absolute harmony with historical researches, as well as with the results of the analyses of Bohemian Sphagnum peats (Sitenský, Rudolph, Firbas). Here I shall give but 2 characteristic examples:

1. The beech wood on the ridge by Svatý Ivan, near Příbram, disclosed the following successions in the course of the last 3 decades:

a. In the year 1898 it had the typical undergrowth of mountain beech forests,

intermingled with *Tilia platyphyllos* and *Acer pseudoplatanus*, both of which trees were later exterminated. In the undergrowth I found still *Prenanthes purpurea*, *Mellitis melissophyllum*, *Lilium martagon*, and *Daphne mezereum*, all of which later died out.

TABLE 2. SOIL CONDITIONS IN THE BRDY

Stages	Forest	Undergrowth	Layer of Humus	Substratum
I.	Mixed and partly also deciduous forests in abundance.	Herbaceous undergrowth relatively abundant.	Humus slightly acid, of considerable thickness, in places even mould.	Podzolation slight.
II.	Mixed and deciduous forests on the retreat; cultivated spruce forests predominate.	Herbaceous undergrowth on the retreat; Vaccinia, Deschampsia flexuosa, Luzula nemorosa well developed; conditions becoming favorable for Calluna vulgaris and Vaccinium myrtillus.	More acid, raw humus being formed.	Podzolation increasing.
III.	Coniferous forests generally predominant; mixed and deciduous forests rare.	Undergrowth of Calluna vulgaris and Vaccinia.	Highly acid raw humus.	Typical podzol profiles; frequent formation of ortstein.

b. In 1916 **Tilia platyphyllos* still grew there, though rarely, and in the undergrowth grew **Asperula odorata*, **Vicia pisiformis*, **Vicia silvatica*, **Corallorrhiza trifida*, **Digitalis ambigua*, **Atropa belladonna*, *Sanicula europaea*, *Lamium luteum*, *Phyteuma spicatum*, *Hepatica triloba*, *Pulmonaria obscura*, *Lathyrus vernus*, *Moehringia trinervia*, *Lactuca muralis*, and *Oxalis acetosella*.

All of these species were found even in the year 1898, except *Viola collina*.

c. In 1925 all the species designated with an asterisk were extinct, while *Vaccinium myrtillus* and *Deschampsia flexuosa*, locally also *Festuca ovina*, had already made their appearance. Of the former species, in so far as they were preserved, mainly *Luzula nemorosa* and *Calamagrostis arundinacea* became abundant, while the true beech types were on the retreat. Simultaneously there came about, through the admission of more light into the beech wood, a disintegration of the soil, which circumstance is favorable to the invasion of the hercynian types from spruce forests.

2. A mixed coniferous forest above the meadow Kardavecká louka near Pičín:

a. In the year 1896–1897 there were growing *Soldanella montana* and *Asperula odorata*.

b. In the year 1902 both these species had already disappeared, but in the forest, devoid of *Fagus silvatica* and *Acer pseudoplatanus*, which undoubtedly once grew there, were to be found *Rosa pendulina*, *Polygonatum verticillatum*, *Knautia silvatica*, *Vicia pisiformis*, *Euphorbia dulcis*, *Mercurialis perennis*, *Lamium luteum*, *Pulmonaria obscura*, *Hepatica triloba*, **Lilium martagon*, **Carex silvatica*, *Carex digitata*, *Poa nemoralis* and others. The species designated with

an asterisk had disappeared by the year 1916. I ascertained 2 further stages in the surrounding forests in the direction of the forest road of Pičín, namely:

c. *Luzuletum nemorosae.*

d. Together with *Luzula nemorosa* there were already growing also *Vaccinium myrtillus* (abundantly) and *Deschampsia flexuosa* (scattered).

e. The last stage, today of most extensive area, is characterized by the complete extinction of the beech types, respectively, of the mixed forest types, and the expansion of the spruce hercynian types. At the same time the soil has deteriorated, the amount of nitrogen present has diminished, the acidity has been augmented, and a more decided podzolation has taken place.

This succession was brought about by anthropic influences, that is, in the first place, by the cultivation of unmixed forests of *Picea excelsa* and *Pinus silvestris*, and also by the partial deforestation of the region, which disturbs the equilibrium of the bio-climatic conditions even in the parts covered by forests.

II. The Evolution of the Vegetation and the Climate in Czechoslovakia Since the Glacial Period

Though no remnants of the diluvial flora were ever found within our territory we nevertheless know from the former extent of the glaciers that the whole interior of Bohemia remained free of ice even when the glaciers were most extensive, so that it formed a refuge, especially for the panonic vegetation of today, in which even forest associations were preserved; undoubtedly even the beech had an isolated diluvial refuge here, far more probably than in the Ceskomoravská vysočina, the Bohemian-Moravian highland, which is acknowledged—with certain reservations—also by Lämmermayer.

Neither were any remnants of the tundra vegetation found on the territory of the Czechoslovak Republic, which is rather surprising, considering the numerous deep Sphagnum peat bogs. There are 2 possibilities:

a. Either they will yet be found in the peat bogs of the Krkonoše (Giant Mountains) or of Sumava (the Böhmerwald).

b. Or the peat bogs of Bohemia originated somewhat later.

A fundamental mistake is the identification of the conditions in Scandinavia and northern Germany with those of central Europe, as was demonstrated by Polish scientists, especially by Szafer, as well as by Swiss scientists. Bohemia, in a certain sense forming an independent climatic whole, had its own special climatic and edaphic conditions, and it is self-evident that simultaneously in the mountains, on the boundaries, and on the warm lowlands (the valley of the Labe and the Ceské Středohoří) the plant associations were absolutely different floristically and ecologically.

The Sphagnum peat bogs of the Rudohoří have been studied in greatest detail (Rudolph and Firbas), also by the pollen-analytical method, used for the first time by Post. Here the succession is as follows:

1. The *Pinus silvestris* period, with the birch and the willow.
2. The *Pinus silvestris* and *Corylus avellana* period: great expansion of *Corylus avellana* on the ridges, the beginning and the maximum of the expansion

of the mixed oak forests (*Ulmus, Tilia, Quercus*), and the beginning of the expansion of the spruce.

3. The period of mixed oak forests and of the spruce. The dominance of the spruce, besides a considerable expansion of the mixed oak forests, and the immigration of *Fagus silvatica* and *Carpinus betulus.*

4. The period of *Fagus silvatica* and *Picea excelsa*: the expansion of the beech, *Picea excelsa* predominating at the beginning. The decline of *Quercus* and *Corylus avellana*, and the immigration of *Abies alba*, which begins to expand toward the end of this period.

5. The period of *Fagus silvatica* and *Abies alba*: the dominance of the beech and the fir on the ridges, while the spruce holds a subordinate place.

6. The present period of *Picea excelsa*: a new expansion of the spruce, due to forest culture, and an accompanying decline of the beech and the fir.

But already the peat bogs of the Bohemian hill country show a certain difference, as was shown by Rudolph and Firbas.

It is very difficult to deduce the character of the climate from the vegetation. This is to be seen clearly from the discussions on the interglacial and postglacial xerothermous periods, in which the opinions of various authors differ diametrically from one another. E. Furrer states with emphasis that in Switzerland the tundras, forests, and steppes existed side by side synchronously.

As regards Bohemia, I give in Table 3 the result of my studies:

TABLE 3. A SCHEMA OF THE EVOLUTION OF THE VEGETATION OF BOHEMIA FROM THE GLACIAL PERIOD TO THE PRESENT DAY.

Period	Vegetation: Mountainous regions	Vegetation: Lowlands	Climate	Remarks
1. Glacial (end of the glacial period).	Arctic-alpine plains, tundras.	Locally coniferous and mixed forests (*Fagus silvatica*) dealpine steppes.	Cold and damp, oceanic.	Alpine elements penetrate into the lowlands of Bohemia; subarctic forests expanded in the river valleys.
2. First Postglacial period.	Pine forests, with birches and willows.	Development of a more xerothermous vegetation as well as herbaceous associations, having their climaxes in the expansion of continuous steppes in the Bohemian Stredohori. in the valley of the Labe, and in the warm parts of central Bohemia.	Gradually warmer and dryer, with a decrease of humidity in summer, more and more continental in character.	Schustler's sarmatic (a, b) and panonic (c) period; Rudolph and Firbas's pine (a), pine and hazel (b) and mixed oak and spruce periods.
a. Pine phase (subxerothermous).				
b. Phase of *Corylus avellana* (xerothermous).	Forests of pine and *Corylus avellana*, expansion of deciduous trees and shrubs (beech excepted).			
c. Oak phase (climatic optimum.)	Mixed oak forests.			
3. Second Postglacial period (Atlantic)		Xerothermous vegetation of steppes and forests gradually declines, but new types are added.	Climate somewhat damper, more oceanic.	Schustler's Atlantic and Baltic periods; Rudolph and Firbas's beech-spruce period (a) and beech-fir period (b).
a. Spruce phase.	Oak on decline, spruce abundant, beech and fir expanding.			
b. Beech and fir phase.	Beech and fir in greatest development.	Deciduous and mixed forests penetrate into the steppe region.		
4. Third post-glacial period.	Beech and fir on decline, spruce again dominant, and in dryer places pine. Mixed forests of beech and fir locally.	Xerothermous deciduous forest associations expanding (besides species of coniferous forests). Open xerothermous associations expanding, but floristically impoverished.	Climate more continental, gradually changing to the climate of today.	Effects of deforestation, settlements, and agriculture ever greater; mountain and mixed forests, which held their own on the hills locally even in the Atlantic period, are decimated through anthropic influences.

Up to the present we have nothing concrete as a basis for the elucidation of the interglacial flora of Bohemia. The Sphagnum peat bogs of Bohemia, which are considered by Sitenský and some other scientists to be partially diluvial, date from the postglacial period. It may be assumed, however, that in the lowlands of Bohemia, which never were covered with ice, there surely grew during the optimal climate of the interglacial period a meridional pontic vegetation, the more delicate species of which probably became extinct, for the most part, during the last great expansion of the ice masses, but some species of which were able to survive till the present day. Beyond doubt the beech was preserved during the diluvial period in Bohemia; indeed, it did not die out even in the western Carpathians, as Pax and Lämmermayer have indicated.

In his important newest paper Wl. Szafer gives a highly interesting account of the interglacial flora of Poland, on the basis of his own studies and of those of Źmuda and A. Kozlowska. He distinguishes 3 regional climatic facies for the second interglacial period, namely the north German, the Silesian-Polish, and the Russian.

III. The Forest-Covering of Czechoslovakia

At the beginning of historical times almost the whole of Bohemia (with the exception of a narrow strip in the valley of the Labe and in the Středohoří) was covered by continuous forests, interrupted in places by marshes and Sphagnum peat bogs.

According to the detailed analysis that I attempted in my latest work (Sborník přírodovědecký Ceské Akademie, 1926), at that time mixed forests predominated almost everywhere, while only in some regions of the mountains, on the boundaries, the highest zone of the mountain forests was formed by unmixed coniferous growths (for instance the spruce forests of Sumava, with an undergrowth of *Athyrium alpestre*). The universal expansion of mixed forests and the predominance of deciduous trees, in comparison with conditions today were the main characteristics of our forests at the time when anthropic influences had but slightly altered the composition of the original virgin forests.

W. Friedrich came to the same conclusion through the analysis of 1232 typical Bohemian topical names, referring to certain trees. The relations are as in Table 4.

TABLE 4. THE NUMBER OF TOPOGRAPHICAL, HYDROGRAPHICAL AND OROGRAPHICAL NAMES REFERRING TO CERTAIN TREES.

1. *Quercus*	225	9. *Fraxinus*	33
2. *Fagus*	186	10. *Ulmus*	22
3. *Betula*	156	11. *Populus nigra*	10
4. *Tilia*	101	12. *Populus tremula*	7
5. *Corylus*	70		
6. *Acer*	61	1. *Pinus*	144
7. *Alnus*	51	2. *Abies*	55
8. *Salix*	43	3. *Picea*	50
		4. *Taxus*	21

Jan Evang. Chadt writes that in Bohemia we have 373 names of settlements referring to deciduous forests and only 109 referring to coniferous forests.

According to the latest statistics the forests in the Czechoslovak Republic cover an area of 4,662,790 hectares or 33.19 per cent of the whole area. An analysis of the forest statistics from the year 1920 leads to the following general conclusions:

1. The forest-covered area varies today within the extremes of 28.82 per cent (Moravia) and 50.6 per cent (Subcarpathian Russia); taken as a whole, 33.19 per cent of our state is covered with forests.

2. Leaving out of consideration the lowlands of southern Slovakia, devoid of forests, we observe that the Carpathian region is much more thickly wooded than the Sudetic-hercynian region; in Subcarpathian Russia, with the wide, continuously wooded strip of the Polonines, the forests cover more than ½ (50.6 per cent) of the entire area.

3. A striking phenomenon is the predominance of coniferous forests in the Sudetic-hercynian region; in Bohemia 85.96 per cent of the forests are coniferous, in Silesia only 62.55 per cent, and in Subcarpathian Russia only 22.33 per cent.

4. In the Carpathian region the deciduous forests cover a far greater portion of the surface. If we take into consideration mixed forests (deciduous and coniferous trees together) as well as pure deciduous forests, we observe a very striking increase from west to east. Thus, in Bohemia the deciduous and mixed forests occupy 14.24 per cent, in Silesia 37.45 per cent, in Moravia 38.98 per cent, in Slovakia 69.42 per cent and in Subcarpathian Russia 77.67 per cent of the entire area actually covered with forests.

5. As regards the individual species of trees, there is to be observed a decrease of conifers in the Carpathian region. If we take into consideration the relative representation of the three main species (spruce, fir, and pine), we find that in Bohemia they cover 89.19 per cent of the entire area actually covered by forests, in Moravia 68.68 per cent, in Silesia 82.18 per cent, but in Slovakia only 39.05 per cent, and in Subcarpathian Russia only 0.03 per cent. The oak is most widely distributed in Slovakia (15.40 per cent), and the beech of course in Subcarpathian Russia (58.79 per cent), though even in Slovakia it is abundant (31.95 per cent).

IV. The Fidelity of Species as a Diagnostic Characteristic of Associations

During the course of my monographical work upon the associations of the Tatra Mountains of Biela, on the basis of several hundred detailed sociological analyses, I could not evade the problem as to which characteristics may be considered distinctive of the individual associations of this mountain vegetation. As an example I have chosen the especially typical calcicole association *Festucetum carpaticae*.[3] This association, which is rather hygrophilous and therefore always without mosses and lichens, is best developed in the ravines on steep mountain slopes between the altitudes of 1500 and 2000 m. It ascends, however, to the highest altitudes (2150 m.) and in places descends quite low, for instance on the water-soaked moss growth with *Heliosperma quadrifidum* near "Sedm

[3] Karel Domin, Festucetum carpaticae in the Tatras of Biela, Bull. de l'Acad. Boh. 1925.

Pramenů" at the entrance of the corrie "Holubyho důl" as low as 1170 m. and on the slopes of Zihlavno above the valley of Cierná voda even to 1070 m. which up till now is the lowest known locality.

In 10 analyses of this association, bearing upon very extensive individual expanses of the association, I ascertained in all 157 species, the classification of which according to stability I carried out on diagrams. Three species occur in all 10 analyses, 4 in 9, 3 in 8, 6 in 7, 14 in 4, 17 in 3, 31 in 2 and 61 only in 1.

The biological spectrum of this association, containing no mosses nor lichens whatever, is as follows:

BIOLOGICAL SPECTRUM ACCORDING TO *RAUNKIAER*

Life-form	Number of species	In per cent
F	1	0.64
CH	10	6.37
H	126	80.25
G	12	7.65
T	8	5.09
Total	157	100.00

The association is sharply defined physiognomically and floristically, as well as ecologically, and the question remains as to how to characterize it. But in what shall we seek its distinguishing features as an association? In the so-called "faithful" species according to the Swiss school or in the constant species according to the school of Upsala? I do not care again to discuss at length this question, which I developed in detail in my book "Problems and methods of plant sociology," Prague, 1923 (on pages 137–141). Not even now can I change my opinion on the faithfulness of species and the value of constancy. Numerous analyses, which I made during the last two years, led me to the conclusion that these faithful species are very hard to find, because as a rule they do not exist. If we are taking into consideration the faithfulness of species, it is evident that we ought to do so with regard to a natural, even if small, region; in our case, then, at least with regard to the whole of the Tatras of Biela. In this way we come to see that at most 2 species of our *Festucetum carpaticae* can be designated as faithful, namely, the dominating grass, which appears in other associations only exceptionally, and *Callianthemum rutifolium*. As for this very rare Tatra plant, it must be remarked that it is a species confined mostly to granite and in the High Tatras growing in an entirely different plant community. Its comparatively abundant occurrence in the corrie between Zdárská Vidla and Havran is surprising in a certain sense, but if we take into consideration the character of this glacial corrie, open to the north, we see that this species could find a very appropriate habitat here. Of course, *Callianthemum rutifolium* was recorded by Fritze and Ilse as very rare also in the lower corrie between Havran and Nový, where, however, it was looked for in vain ever since that time. In which association it grew here it is hard to decide, though it is very unlikely that it was in the *Festucetum carpaticae*. Consequently the faithfulness of even this species is of problematic value for our association. It cannot be said of any one of the other species that it is confined to this association, and so we have no species

whatever entirely faithful to this association except *Festuca carpatica*. This fact, which holds true for most of the other associations, leads me not to consider the faithful species as the characteristic of the association, but to describe and define every association from more points of view, in the first place, with regard to the constancy of species. Of course constancy alone is not always sufficient. Thus, in our case I consider as a distinguishing feature of the association the presence of the rare Tatra limestone papilionaceous species, even though a number of them are only accessory species with regard to their constancy. Only *Hedysarum obscurum* has a high degree of constancy (70 per cent); *Astragalus frigidus* occurs in 4, *A. alpinus* in 3, *Oxytropis sericea* in 2, and *Astragalus oroboides* in 1 of 10 analyses. Also the occurrence of species of the genus *Erigeron* has its significance, even though in one case it be *E. atticus*, in the second case *E. alpinus*, and in the third *E. neglectus*. Negative characteristics are always of great importance, and of the positive ones especially the group of species regularly accompanying the association is of weight. The distribution of life-forms also has its diagnostic value, but of course it would have to be carried out into greater detail than the fundamental classes of Raunkiaer.

For the evaluation of the faithful species I refer to the valuable paper of the prominent Polish geobotanists Szafer, Pawlowski, and Kulczynski "Die Pflanzenassoziationen des Tatra-Gebirges.—I. Teil: Die Pflanzenassoziationen des Chocholowská-Tales," which in a certain sense may be regarded as a fundamental work on the modern sociological classification of the Tatra vegetation. The authors are entirely convinced of the value of the faithfulness of species and write with emphasis: "Als Assoziationen gelten für uns nur jene Pflanzengesellschaften, die durch mehrere in ihnen ausschliesslich oder doch hauptsächlich vorkommende Arten, ihre Charakterarten gekennzeichnet werden. Je höher die Anzahl derselben ist und einen je höheren Treuegrad sie zeigen (bestandestreu, -fest, -hold), desto ausgeprägter und leichter erkenntlich erscheint die betreffende Assoziation. Pflanzengesellschaften ohne jedwede Charakterarten werden konsequenterweise nur als 'Varietäten,' 'Nebentypen' oder 'Facies' bestimmter wohl charakterisierter Assoziationen bezeichnet."

It is, of course, evident that the conception of faithfulness for a single valley is entirely fallacious, even absurd. It is necessary to take into consideration the whole limestone—and possibly the granitic—region of the Tatras, according to what association we are analyzing.

The authors use for the designation of the degree of faithfulness the usual C_1, C_2, C_3. Thus, for instance, Piceetum excelsae has the following for the associational characteristic species:

C_1–C_2: *Lycopodium annotinum* (in one of ten analyses).

C_2–C_3: *Picea excelsa, Luzula silvatica, L. flavescens, Pirola uniflora, Lycopodium selago.*

The first species is to a considerable degree (though not absolutely) faithful to this association and to some related ones. But already *Picea excelsa* can be designated, at most, as faithful to the respective association-group; *Lycopodium selago* grows in the most various mountain, subalpine, and alpine associations;

Luzula silvatica occurs also in a number of associations, accompanies *Mughetum montanae* with considerable constancy, and is recorded by the authors as a characteristic species of the association *Calamagrostidetum villosae*, and the same holds true for *Pirola uniflora*. And in this way we could go through one association after the other and convince ourselves that "les espèces caracteristiques" or the so-called faithful species, in reality, are not what they are called. Thus *Calamagrostidetum villosae* has according to the authors three faithful species, namely, *Poa Chaixi* (C_2), *Gymnadenia albida* (C_2), *Luzula silvatica*(C_3); *Vaccinietum myrtilli* also three species, *Gentiana punctata*, *Melampyrum pratense* and *Vaccinium myrtillus* (the last C_5); *Pinetum montanae* only 2 species, *Pinus pumilio* and *Athyrium alpestre*. Except for *Pinus montana*, however, all these species are represented in different Tatra associations, so that it is not possible to speak of their restriction to a single association. And thus we can analyze one association after the other and convince ourselves that the classification of associations according to species confined or almost confined to them is all very good theoretically but impracticable in reality, because most associations have no such faithful species, or at most a single species.

In my opinion constancy furnishes a better basis. Of course I would always emphasize those accessory species that even with a low degree of constancy are characteristic for the association in this or that regard. It is not possible to generalize as to which these species are, because it is necessary to judge each association individually.

I was the more convinced of the correctness of my opinion when I compared an additional analysis of *Festucetum carpaticae* with the results of the 10 other analyses shown above. It is an analysis of a Festucetum on Murán, at an altitude of 1500 m., on a slope with a northern exposure.

This *Festucetum carpaticae*, although physiognomically exceedingly characteristic, looked to me like an atypical and somewhat more hygrophilous example of this association. If, however, we compare its analysis with the general result of the analyses of this association in different parts of the Tatras of Biela, we find that in reality it is quite typical, for it has almost all the constant species, that is, all 3 species with a constancy of 100 per cent, 3 species out of 4 with a constancy of 90 per cent (*Polygonum bistorta* lacking), and 2 species out of 3 with a constancy of 80 per cent (*Crepis mollis* lacking). It is interesting to note that not even 1 single new species appears. The curve designating constancy descends also at 50 per cent and of course rises but little in the lowest class, as is natural on account of the small number of accessory species. The same is to be observed on the curve designating the distribution of species.

As a second example I shall bring to notice an extremely uniform and monotonous association, *Nardetum strictae*, which I analyzed by the quadrat method in the mountains Ceskomoravská vysočina (Czechomoravian highland).[4]

Twenty analyses contain in all 50 species, namely, 47 phanerogams and 3 cryptogams. In considering the stability of the species of this association, we

[4] See the detailed analyses in my book "Rostlinná Sociologie," 1923, pages 251–272, with accompanying tables.

can eliminate beforehand all species that were ascertained only once as accidental. These species are comparatively very numerous, 20 in all, as follows:

1. *Briza media*,
2. *Carex pallescens*,
3. *Carex stellulata*,
4. *Carex panicea*,
5. *Juncus squarrosus*,
6. *Juncus filiformis*,
7. *Hieracium auricula*,
8. *Gnaphalium silvaticum*,
9. *Hypochoeris radicata*,
10. *Centaurea jacea*,
11. *Succisa pratensis*,
12. *Solidago virga aurea*,
13. *Pimpinella saxifraga*,
14. *Rumex acetosa*,
15. *Rumex acetosella*,
16. *Hypericum quadrangulum*,
17. *Lotus corniculatus*,
18. *Campanula rotundifolia*,
19. *Pedicularis silvatica*,
20. *Potentilla procumbens*.

Among these entirely accidental species is an especially large number of Compositae. It is not surprising that foreign elements invade this or that association from the neighborhood, and least surprising in the case of Nardeta, which settle secondarily on soil that is dry and sterile, for instance, forest clearings or Sphagnum meadows that are drying out. In the above list may be recognized some species that belong to the vegetation of damp meadows, namely, numbers 3, 4, 6, 7, 11, 14, and 19, and further some species that occur in our region mainly on grassy field-borders and flowery meadows (1, 9, 10, 13, 17), and also species belonging to forests (20) or clearings (2, 8, 12, 16, 14) and 1 species (18) that grows both in drier forests and in meadows. Of course, often these foreign elements, having different ecological requirements, remain only in a sterile state, are backward in development, or succeed only in producing seedlings, which subsequently perish. *Juncus squarrosus* alone, although it often grows on damper ground, prospers under the same conditions as *Nardus stricta*, and probably would appear oftener in Nardeta if it did not occur only as a very rare species in the region in question.

Eight other species were found to occur twice, namely:

21. *Deschampsia caespitosa*,
22. *Cynosurus cristatus*,
23. *Carex Goodenoughii*,
24. *Luzula campestris*,
25. *Luzula nemorosa*,
26. *Hieracium vulgatum*,
27. *Leontodon hispidus*,
28. *Vaccinium vitis idaea*.

Of these species 2 (21, 23) are more hygrophilous, though of course in one case *Deschampsia caespitosa* developed an interesting subxerophilous form, and 2 others (26, 28) are invaders from the forest. Two species (22, 27) grow scattered on field-borders and mesophilous meadows, while *Luzula campestris* occupies various localities. Surprising is the occurrence of *Luzula nemorosa*, always as small, undeveloped, sterile individuals, which I never found anywhere in forests or on clearings in this district. Kovář, 1909, mentions it as present in the region beginning with the warm slopes of the valley of the Sázava River, from Nýdek to Peperek. Inasmuch as I found it a number of times, its occurrence in this association after all has a certain significance, especially with regard to the fact that this otherwise universally occurring hercynian and heath

species is completely or almost completely absent from the forests of the region in question.

To these 28 species must be added 5 others, which occur in 3 diagrams out of 20, namely:

29. *Chrysanthemum leucanthemum*,
30. *Vaccinium myrtillus*,
31. *Plantago lanceolata*,
32. *Ranunculus acer*,
33. *Viola canina*.

Of these species 1 (30) is a forest species, 1 (33) is a forest and heath meadow species, and the other 3 are meadow species.

I shall group the rest of the species, with a higher coefficient of stability, in a convenient table, Table 5.

TABLE 5. ADDITIONAL SPECIES OF THE ASSOCIATION NARDETUM STRICTAE

In 20 diagrams, number of times occurring		Resulting coefficient of constancy in per cent
34. *Cladonia*	5	25
35. *Festuca rubra*	} 6	} 30
36. *Antennaria dioica*		
37. *Polygala vulgaris*	4 } 7	20 } 35
38. *Polygala oxyptera*	3	15
39. *Agrostis vulgaris*	} 8	} 40
40. *Festuca ovina*		
41. *Calluna vulgaris*		
42. *Veronica officinalis*	9	45
43. *Carex pilulifera*	10	50
44. *Hieracium pilosella*	} 11	} 55
45. *Deschampsia flexuosa*		
46. *Polytrichum juniperinum*	13	65
47. *Hypnum Schreberi*	14	70
48. *Danthonia decumbens*	15	75
49. *Potentilla tormentilla*	16	80
50. *Nardus stricta*	20	100

If we classify all 50 species according to the coefficient of stability into 10 groups, each group having a range of 10 per cent, the classification appears as in Table 6:

This classification of the species according to stability stands out even better in a graphical representation.

TABLE 6. CLASSIFICATION OF SPECIES ACCORDING TO STABILITY, INTO TEN GROUPS

0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–100
20	13	1	4	4	2	1	2	1	1
no. 1–20	no. 21–23	no. 34	no. 35–38	no. 39–42	no. 43–44	no. 46	no. 47–48	no. 49	no. 50

These tables clearly show us the stability of the species on the basis of 20 investigated quadrats, which number I consider sufficient because of the uniformness of the association. In the first place we see that a majority of the species (33 out of 50, which means fully 66 per cent) is crowded into the lower groups (5–15 per cent), which phenomenon occurs regularly also in other associations. These species are accidental, with little sociological significance, and some

sociologists (see Rübel) simply leave them out of further consideration. Next follows a decided hiatus, then 1 species appears, with a stability of 25 per cent, and after that the species are more and more numerous to 40 per cent. From 40 to 80 per cent the species on the whole are uniformly scattered, and after an empty space of 20 per cent comes the dominating species of the association.

The Upsala school designates as stable to the association only the species with a stability of more than 90 per cent, according to which the Nardeta of the Czechomoravian highland would possess their only stable species in their dominating grass. The Swiss sociological school designates as stable the species with a stability of 80–100 per cent, according to which also *Potentilla tormentilla* is stable. Usually many species are crowded into the lowest and highest groups, which, however, is not the case with our association. Therefore, the question arises whether in the case of Nardeta the area of 1 square meter is not smaller than the minimal area of this association, and whether by trying a larger area (2–4 square meters) we would not obtain a diagram that would correspond to the phenomena of stability in other associations.

In my opinion the chosen size of one square meter is sufficiently large, as I convinced myself that even with an area of 4 square meters in growths of *Nardetum strictae*, otherwise typical, one can find areas of this 4-fold size from which some species with a stability of 55–80 per cent are absent—some species which we might be inclined to look upon as stable to the association. Of course, it would be possible to find hundreds of square meters in which all these species are represented, and which would raise the stability of all of them to 100 per cent. However, I purposely avoided selecting quadrats that would correspond to definite assumptions made beforehand, and always chose typical places in the growth of *Nardus stricta*, never changing the place of the quadrat even when I ascertained that very near the outside of its limits were species regularly occurring in Nardeta but not contained within my square. The result obtained in this manner seems to me to be more objective, because it represents to us the growths of *Nardus stricta* exactly as they occur in natyre. From the results of these analyses it is clear to me that with the exception of the lowest groups, in which are crowded together species that are really accidental, the coefficient of stability has not the same significance in all associations. For an association so poor floristically and so monotonous physiognomically the so-called accessory species (with a stability of 40 80 per cent) have a great significance. This association, in which only 4 phanerogams are represented, counting *Nardus stricta*, often shows a tendency to be absolutely dominated by *Nardus stricta*, and in such cases, of course, different plants often present in other parts of the growth may be absent even from large areas. However, exactly in this circumstance appears an individual trait of this characteristic association, which is exclusive in a high degree. Therefore the stability of species in different associations must not be judged by an ironclad rule, which fact our analyses display very conclusively.

Therefore, on the whole, I conclude this chapter as follows:

1. The stability of species offers a far safer guide for the characterization of associations than the fidelity of species.

2. The fidelity of species must be judged with regard to whole natural districts, and not according to only parts of them.

3. Different associations behave in very different ways as regards characteristic species (5 exclusive, 4 elective, 3 preferrent, according to Braun-Blanquet). Some associations, otherwise very characteristic, possess no such species whatever. In such cases a group of species with a relatively high degree of stability is usually the best guide for the characterization of the association.

4. There are no absolutely faithful species in the associations of the Czechoslovakian vegetation.

V. Decomposing Stumps and Trunks as Points of Support for the Invasion of Calcifugous Elements into a Calcareous Region

It is a well known fact that a characteristic community of lichens and mosses settles on decomposing stumps, irrespective of the geological substratum on which the stumps grow, the habitat being determined exclusively by the species of the tree. So it happens that in a calcareous region we not infrequently find on the rotting stumps and trunks of spruces micro-associations of sporophytes that otherwise are characteristic of regions with a silicious substratum. However, up to the present the significance of these habitats for the penetration of calcifugous elements into a calcareous region was never properly emphasized. I investigated these conditions in the Tatras of Biela and ascertained that *the significance of decomposing stumps and trunks is far-reaching not only for sporophytes, but also for spermatophytes*, and that it offers the explanation for many surprising localities and even of whole colonies of non-calcicolous plants on a calcareous substratum. In primeval forests, where there are many decomposing trunks, extensive growths of calcifugous plants are thus enabled to gain a foothold and permanently to change, through the formation of acid humus (for example Vaccinia), the character of the habitat, and of the whole growth comprised therein. When the trunks are completely decomposed, it looks as if these intruding groups of plants were growing under the same soil conditions as the surrounding undergrowth, more or less calcicolous in character. In reality, however, this is not the case, as I have had the opportunity of convincing myself through detailed study in many places.

If we ascend, for example, from Podspady above the gulch of the brook Nový to the lower corrie between Havran and Nový, we observe in the highest forest zone still the typical undergrowth of mountain forests on a calcareous substratum. At an elevation of about 1260 m., however, I observed on a spruce stump in an advance state of decomposition an interesting allogenous community, localized exclusively on this stump and essentially different from the surrounding undergrowth. Especially, club-mosses grew here in great abundance (very profusely, *Lycopodium annotinum*; profusely, *L. selago*, also *Aspidium spinulosum*, *A. dryopteris*, *Oxalis acetosella*, and some mosses—*Dicranum* sp., *Hylocomium splendens*, and others), while whole areas were covered with lichens,

namely, *Icmadophylla ericetorum* (abundant), *Cladonia squamosa*, *Cladonia digitata*, *Cladonia cenotea* (abundant), and *Cladonia ochrochlora*.

In the lower forest zone (below 1000 m.) I observed this community in Cierná dolina, for example, where in the forests there is a select calcicolous undergrowth, but where on the moss-grown, decomposing stumps there is settled a characteristic community of lichens, namely: *Cladonia Floerkeana*, *C. gracilis*, *C. squamosa*, *C. ochrochlora*, *C. macilenta*, *C. Botrytis*, *C. digitata*, *C. pleurota*, and the mosses *Dicranum scoparium* and sometimes less abundantly *Hypnum Schreberi*; on the vertical (drier) sides of the stumps grow *Xylographa abietina*, *Lecanora subravida*, and *L. symictera*. Occasionally even Polytricha settle on these stumps, also cranberries (*Vaccinium vitis idaea*), and once I found even heather (*Calluna vulgaris*).

In the mountain corrie between Zdárská Vidla and Havran I observed in the zone between 1160 m. and 1250 m. that a single rotting spruce stump, on an open grass growth with a very characteristic, exclusively calcicolous vegetation, was all that was necessary for the formation of a whole colony of *Vaccinium vitis idaea*, with *V. myrtillus*, *Potentilla aurea*, and *Luzula nemorosa*. In the course of time the stump will disappear entirely, of course, but there will remain the acid humus, to the formation of which both Vaccinia contribute substantially, and it is probable that this oasis of calcifugous species will hold its own, just as at a height of around 1600 m. in the same corrie very low dwarf pines, lying close to the ground and forming on the barren only small groups (with a scattering of bushy spruce) have sufficed to attract to this calcareous substratum, covered, however, with acid humus, such species as *Aronicum Clusii*, *Gentiana punctata*, *Salix retusa*, and *Vaccinium vitis idaea*.

Still more surprising are carpets of Sphagna, which we find in places on calcareous substrata or even directly on calcareous rocks. An explanation of this remarkable phenomenon was afforded me in Bielovodská dolina beyond Bílá polana, where above the road, which follows the stream, there rises a calcareous cliff where a long, thick carpet of *Sphagnum* hangs directly from the calcareous rock, in the immediate neighborhood of calcicolous species (*Saxifraga aizoon*, *Aspidium Robertianum*, and others). Upon closer investigation, however, one discovers that this growth of *Sphagnum* surrounds a half-decomposed spruce trunk (or thick limb) hanging from the rock. It appears, though, that for such an occurrence of *Sphagnum* a necessary condition is extreme dampness, which is exactly what characterizes this valley with a northern exposure, above a mighty mountain torrent.

Also in the narrow, rocky gorge of Nový's Brook, which penetrates deep into the forest zone and is extraordinarily damp and shady, at a height of 1160 m. *Sphagnum* appears on the calcareous rock with the first dwarf pines, hanging down in immense pillows. On account of the verticalness and inaccessibility of the rocky cliffs I could not investigate this locality with thoroughness, but higher up in the forest above the gorge I also found carpets of *Sphagnum* and was able to ascertain that in every case decomposing stumps or trunks were present.

If we ascend still higher into the forest in the direction of the hunters' path that leads to the beginning of the lower corrie between Havran and Nový, we find in the spruce forest, at an elevation of 1205 m.–1218 m., the first specimens of *Pinus cembra*, old and well-formed trees. But exactly here, in the proximity of *Pinus cembra*, we are surprised by the undergrowth of the wild spruce forest, preponderantly of hercynian character, which is unusual for a calcareous substratum. The composition of this "hercynian" forest association, limited to this locality, is in the main as follows:

Vaccinia predominate, *Vaccinium myrtillus* being more abundant than *V. vitis idaea; Homogyne alpina* is very abundant, *Deschampsia flexuosa* is abundantly scattered, *Melampyrum silvaticum* is scattered, and *Prenanthes purpurea* occurs singly. On the whole, the growth is mossy, but the mosses present are only the ordinary hercynian mosses, especially *Dicranum scoparium* and *Hypnum triquetrum*, while *Hylocomium splendens* and species of *Polytrichum* occur less frequently. I am convinced that this community is an edaphic consequence of the quantity of decomposing trunks present. There are even whole piles of entirely rotten wood lying about that are overgrown with mosses and some vascular plants.

It would be possible to give a large number of similar cases, which are noteworthy for the reason that they elucidate to us the manner, until now overlooked, in which calcifugous species can invade calcareous regions.

VI. The Distribution of Plant Communities in the District of Libusin and Their Relation to the Edaphic Factors

1. This district of central Bohemia, the well-known centers of which are Smečno, Libušín, and Kladno, has a very striking character owing to its geological structure. Everywhere are to be seen long table-lands of marly limestone, often plateaus of considerable extent, that usually end abruptly with steep slopes falling into the surrounding country, which for the most part is cultivated, but in places also covered with forests. Only in the lower parts the sandstone that everywhere forms a basis for the marly limestone appears at the surface and forms hillocks more or less rounded in shape, instead of table-like hills.

The plateaus themselves are always without water, as the weathered marly limestone allows the rainwater to soak through easily. Beneath the marly limestone lies a layer of clay, impermeable to water, and lower down strata of sandstone and clay alternate, with here and there a thin stratum of conglomerate (Fig. 2). At a considerable depth, between 400–450 m., a stratum of coal appears which is mined.

2. The rainwater, falling as pure water on the expanses of marly limestone, dissolves compounds of calcium; then, as water containing calcium carbonate, quickly soaks through the weathered marly limestone into the substratum of silicious sandstones, which forms the basis for the marly limestone, and renders it nutritious and calcareous, thus making possible the local occurrence of calcicolous associations. The main springs are to be found, of course, at the meeting-place of the marly limestone and the clay.

3. The underground water soaks downward in a curve toward the borders of the plateau, for which reason the borders of these marly limestone plateaus are driest, the underground water being deepest beneath them.

4. With the course of time the marly limestones on the surface have been leached out, so that they scarcely effervesce when strong acid is poured on them, and their weight is hardly half of the weight of marly limestone that has not been leached out.

The marly limestone plateaus are not covered by any non-calcareous sediment that could be considered capable of neutralizing the effect of their chemical properties. The fine marly limestone debris is present at the very surface, being covered only by a thin layer of raw humus. The permeability to water of marly limestone, and the intensive leaching out connected with it offer an explanation of the undoubtedly surprising fact that all such marly limestone plateaus are covered with a hercynian growth, the borders supporting only a *Vaccinium vitis idaea* community or a *Calluna vulgaris-Vaccinium vitis idaea* community (with *Pinus silvestris* or *Betula alba*).

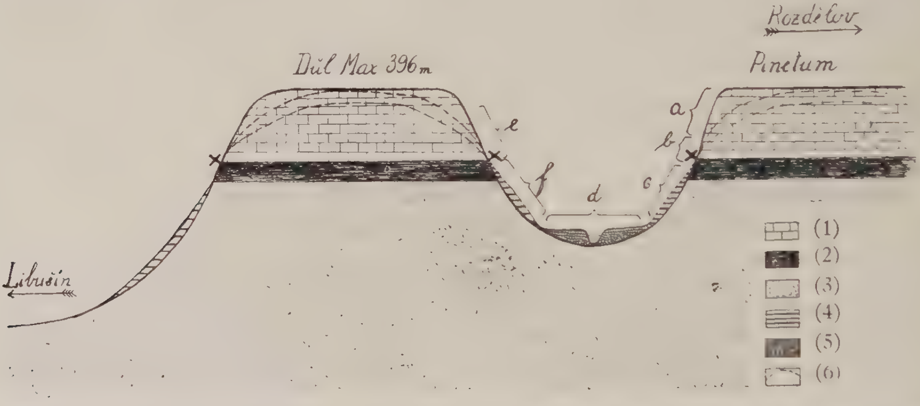


Fig. 2. Profile near Lisbušín (for explanation see text). (1), Marly limestone; (2), Clay; (3), Sandstone; (4), Debris, marly limestone; (5), Soil; (6), Underground water.

5. The boundaries of the calcicolous vegetation proper to marly limestone and the hercynian association is extremely sharp in places where the plateaus end abruptly in steep slopes. Where the plateau falls gradually the boundary is less sharp, because the marly limestone debris is carried down along the slope and the leaching out is only partial.

6. The sandstones make their influence, as a silicious substratum, felt on the vegetation only in places where calcium-carbonate-bearing water does not soak into them, or where they are not covered with marly limestone debris. The calcareous nature of the water even in the sandstone district makes itself apparent in places through the gregarious occurrence of *Chara foetida*.

7. The waters running out of marly limestone do no more leaching out, being saturated, but on the contrary deposit lime, as we know from the frequent occurrence of incrustations.

8. On the marly limestone slopes may be distinguished two and in some cases three zones: the lowest is naturally the dampest (type *Astrantia*), the intermediate is occupied by mesophytic deciduous woods, and the highest is more xerophilous in character, being represented differently on the drier slopes (type *Dictamnus-Sorbus aria*) than on the slopes covered with more humus (type *Aconitum vulparia*).

9. As regards associations, the following general statements may be made:

a. The hygrophilous to mesophilous meadow associations are represented but very poorly, and for the most part the meadows are semi-natural.

The wet meadows have preserved their vegetation better. I saw a good example of this kind of meadow in the valley between the military shooting-ground and the open-air theater. It belongs to the type *Scirpus silvaticus-Holcus lanatus* and has the following composition:

Scirpus silvaticus	8	*Eriophorum angustifolium*	2
Holcus lanatus	6	*Luzula campestris*	3
Ranunculus acer	5–6	*Trifolium pratense*	3
Cardamine pratensis	5	*Angelica silvestris*	3
Carex Goodenoughii	4	*Heracleum sphondylium*	2
Lychnis flos cuculi	4	*Trollius europaeus*	2
Equisetum palustre	4	*Colchicum autumnale*	1–2
Rumex acetosa	4		
Cirsium canum	4		

b. The xerophilous and subxerophilous grassy associations are developed on the marly limestone field-boundaries and in situations more open, among bushes. These growths are secondary, but are formed of species indigenous to the region, and today they show more or less stability. Semi-xerophilous types are represented by meadows of the type *Avenastrum pubescens*. As an example I give 2 analyses:

On the slope immediately below Max's mine there are among the bushes on the marly limestone open grassy places, locally not completely closed, that are dominated by *Brachypodium pinnatum*, in which the second place is held by a gregarious growth of *Carex humilis*, and the third place by *Festuca sulcata*, occurring very abundantly. *Anthoxanthum odoratum* and *Carex glauca* are also very abundantly scattered, as well as *Coronilla vaginalis*, and *Chrysanthemum corymbosum*, while here and there occur *C. leucanthemum*, *Primula officinalis* (in a small form, in accordance with the ecological conditions of the habitat), *Linum catharticum*, *Lotus corniculatus*, and others.

On clay soil these growths acquire a somewhat different character, as we may observe, for instance, on the bare southern slope of Max's valley. Here, in the middle portion, the substratum is formed by clay or sandstone, but these materials are almost everywhere covered by soil brought down from the marly limestone covering of the slopes lying higher up, and into these water containing calcium carbonate flows from springs originating on the boundary of the clay. For this reason the meadow (semi-steppe) associations here have the character of the associations belonging to argillaceous and calcareous soils.

The grasses are not altogether predominant, but nevertheless take an important part in the formation of the growth, the species being *Festuca sulcata*, *Brachypodium pinnatum* and *Poa pratensis*. Associated with them are the following:

Tetragonolobus siliquosus	7	*Bupleurum falcatum*	3
Salvia verticillata	8	*Potentilla opaca*	3
Thymus praecox	8	*Lotus corniculatus*	3–4
Thymus Bayeri	3	*Linum catharticum*	5
Salvia pratensis	5	*Pimpinella saxifraga*	3
Reseda lutea	3	*Sanguisorba minor*	3
Centaurea rhenana	3	*Euphorbia cyparissias*	3–4
Scabiosa ochroleuca	3	*Hieracium pilosella*	3
Ononis spinosa	3		

c. The steppe Festucetum vallesiacae is a very characteristic association, which of course, however, is confined to basalt. It is well developed on the top of Vinařická hora (410 m.) on the slope turned toward the south, that is, southwest. Its aspect here is purely grassy, and on an analyzed area of 16 square meters *Festuca vallesiaca* has a dominance of 100 per cent, while *Thymus Löxyanus* var. *stenophyllus* is scattered comparatively abundantly, and also *Potentilla arenaria* (in a small form) is abundant; in places *Medicago minima* appears, and only very infrequently, *Eryngium campestre*.

As is evident, this association is highly exclusive, and in this respect comparable to Nardetum, the habitats of which, of course, are of an entirely different nature. In the further continuation of the Festucetum described, *Koeleria gracilis* and *Carex humilis* appear on rocky ground.

d. Heaths (Callunetum vulgaris) on leached out marly limestones. *Calluna vulgaris* is a light-loving type and occurs gregariously on clearings and in open birch or pine woods, in which the undergrowth is but very little influenced by the trees. It also forms growths on the open spaces above the railroad track. The heaths are very poor floristically, and are usualy acompanied by *Vaccinium vitis idaea*, *Deschampsia flexuosa*, *Festuca ovina*, *Luzula campestris*, *Antennaria dioica*, *Galium silvestre*, *Chamaebuxus alpestris*, etc.

e. The heaths growing on sandstone have a somewhat different character. In places *Anthericum liliago* grows in them in abundance, and even sand-loving types like *Thymus angustifolius* and some xerothermous types like *Centaurea rhenana*. In the shade of pine forests *Calluna vulgaris* dwindles away or disappears altogether and then in places we observe an inclination to form psammophilous associations with *Corynephorus canescens*, *Helichrysum arenarium*, *Thymus angustifolius*, *Spergula Morisonii*, *Veronica prostrata* and others.

f. Of the forest and bushy formations the following deserve notice: Wet deciduous woods (with *Acer pseudoplatanus*, *Fagus silvatica* and *Alnus glutinosa*) with a rich undergrowth of hygrophilous herbaceous plants like *Crepis paludosa*, *Chaerophyllum hirsutum*, *Astrantia major*, *Ranunculus lanuginosus*, *Paris quadrifolia*, *Filipendula ulmaria*, *Cirsium oleraceum*, *Asarum europaum*, *Pulmonaria obscura*, and *Hepatica triloba*.

g. Damp deciduous woods with an undergrowth of *Astrantia*, with which comparatively few species are intermingled, most often *Lilium martagon* and *Polygonatum multiflorum*.

h. Deciduous woods with humus (*Acer pseudoplatanus*, *Fagus silvatica*, and *Tilia parvifolia*), containing a very rich herbaceous undergrowth with the following composition (according to a number of analyses):

Very common or gregarious: *Mercurialis perennis*, *Actaea spicata*, *Aquilegia vulgaris*, *Pulmonaria obscura*, *Melica nutans*, *Myosotis silvatica*, *Primula officinalis*, *Hepatica triloba*, *Lilium martagon*, *Galium silvaticum*, *Urtica dioica*.

Common or fairly common: *Stellaria holostea*, *Melittis melissophyllum*, *Asarum europaeum*, *Prenanthes purpurea*, *Rubus saxatilis*, *Chrysanthemum corymbosum*, *Lamium luteum*, *Veronica chamaedrys*, *Tussilago farfara*, *Galium aparine*, *Glechoma hederaceum*, *Fragaria vesca*, *F. elatior*.

Local: *Cypripedium calceolus*, *Aconitum vulparia*, *Phyteuma spicatum*, *Carex montana*, *C. silvatica*, *Lathyrus vernus*, *Majanthemum bifolium*, *Convallaria majalis*, *Ajuga genevensis*.

Scattered: *Bromus asper*, *Dactylis Aschersoniana*, *Vicia silvatica*, *Inula conyza*, *Viola Riviniana*, *V. mirabilis*, *Stachys officinalis*, *Lactuca muralis*, *Arabis hirsuta*, *Scrophularia nodosa*, *Nephrodium dryopteris*, *N. filix mas*, *N. spinulosum* (*N. dilatatum* as well as *N. elevatum*), *Athyrium filix femina*.

i. Deciduous woods of a more xerophilous character with *Aconitum vulparia* predominating, and also with *Nephrodium Robertianum*, *Lithospermum officinale*, and others.

k. Shrubby growth (*Corylus avellana*, *Cornus sanguinea*, *Carpinus betulus*, *Ligustrum vulgare*, *Crataegus sp.*, *Lonicera xylosteum*, *Viburnum opulus*, *Tilia platyphylla*) displaying in shady places the types proper to deciduous woods, and in lighter places more xerophilous types. Of the more noteworthy species I may name *Cypripedium calceolus*, *Orchis purpurea* (both these orchids are constantly becoming scarcer), *Cephalanthera alba*, *Hierochloe australis*, *Clematis recta*, *Melittis melissophyllum*, *Lilium martagon*, *Ranunculus lanuginosus*, *R. auricomus*, *Stellaria holostea*, *Primula officinalis*, *Aquilegia vulgaris*, *Melica nutans*, *Actaea spicata*, *Lamium luteum*, *Pulmonaria obscura*, *Hepatica triloba*, *Asarum europaeum*, *Rubus saxatilis*, *Galium silvaticum*, *Chrysanthemum corymbosum*, *Veronica chamaedrys*, *Moehringia trinervia*, *Polygonatun multiflorum*, *Convallaria majalis*, *Majanthemum bifolium*, and in lighter places also *Cirsium eriophorum*.

A special community is represented by the xerophilous shrubby growth on the slopes with *Sorbus aria* (besides *Ligustrum vulgare* and *Cornus sanguinea*, also solitary *Juniperus communis*), with *Dictamnus albus* very plentiful, and with *Coronilla vaginalis*, *Anthericum ramosum*, *Teucrium chamaedrys*, *Thlaspi perfoliatum*, *Rubus saxatilis*, *Thymus praecox*, *Polygala amara*, *Cirsium acaule*, and others.

The hercynian forest associations (on sandstone or washed out marly limestone):

1. Very open forests of *Pinus silvestris* and similar birch woods with a heath undergrowth (*Calluna vulgaris*) or with an undergrowth of *Calluna* and *Vaccinium vitis idaea*, which last predominates in places along with *Festuca ovina* and *Deschampsia flexuosa.*

2. Closed pine forests with *Vaccinium vitis idaea* predominating, and with a small number of other xerophilous species.

3. Pine woods with an undergrowth of *Myrtilleta* (a less xerophilous type).

4. Pine forests (and forests containing also other cultivated trees, as well as oak woods and larch woods) with an undergrowth of subxerophilous grasses (*Deschampsia flexuosa*, *Festuca ovina*).

5. Hercynian forests (mainly pines and spruces) with a more abundant undergrowth of grasses and herbaceous plants as a more mesophytic type.

These forest types are of course connected with each other by various transitions. Their undergrowth, on the whole, is very monotonous and characterized negatively by the absence of all species proper to marly limestone. Of the more interesting types from the pine forests may be named *Peucedanum oreoselinum*, *Arnica montana*, *Chamaebuxus alpestris*, *Dianthus silvaticus*, *Galium rotundifolium.* Remarkable is the isolated occurrence of *Prenanthes purpurea* in the dry pine forests. Of rare occurrence is also *Moneses grandiflora.* Of the grasses, *Deschampsia flexuosa* and *Festuca ovina* occur everywhere, here and there *Luzula nemorosa*, *L. campestris* and *Carex montana.* On the heaths is scattered *Genista germanica.* Even on the clearings, in so far as they are not occupied by *Calluna vulgaris* or Vaccinia, only a poor vegetation is developed, with *Hieracium pilosella*, *Veronica officinalis*, *Gnaphalium silvaticum*, *Viola canina*, *Fragaria vesca*, *Antennaria dioica*, *Scrophularia nodosa*, *Brunella vulgaris*, *Epilobium angustifolium*, etc. Infrequently appear *Potentilla tormentilla*, *Epipactis latifolia*, *Platanthera bifolia*, *Ramischia secunda*, *Galium silvestre*, *G. boreale*, *Anemone nemorosa.*

10. The naturalness of the forest associations. As everywhere else in central Europe, we must take into consideration also here the far-reaching anthropic influences on the original plant covering. The marly limestone and sandstone district of Smečno-Kladno nevertheless has retained many of its spontaneous associations, although the country all around is cultivated. On marly limestone that has not been washed out, in places not too much exposed to the sun, the original plant covering was undoubtedly mixed forests with *Acer pseudoplatanus*, *Tilia platyphyllus* and *Fagus silvatica*, and with a very rich undergrowth, many of the elements of which would seem to have accompanied the beech, although one of the most faithful companions of the beech, *Asperula odorata*, is entirely absent here, to all appearances. In spite of the low elevation (around 400 m.) of this district, some mountain elements are present, for example *Vicia silvatica* and others; in fact, it may be said that whole associations of mixed deciduous woods on marly limestone have a mountain or submountain character. After the partial felling of the forests the shrubby vegetation, which previously formed a layer in them, increased. In this way the habitat became drier and warmer, and the expansion of subxerophilous types was made possible.

The zonal differentiation of associations depends purely on edaphic factors, as is demonstrated by the schematic profile of the slope above the shooting-ground (Fig. 3), where the following zones may be seen (from bottom to top):

I. Wet deciduous woods by the stream. Substratum earthy. Underground water near the surface.

II. Damp deciduous wood with Astrantietum on the lowest part of the slope. The substratum of cretaceous sandstones is covered with earth mixed with marly limestone debris.

III. Deciduous wood containing humus, with a rich mixed undergrowth. The substratum sufficiently moist but already considerably drier than the former, namely: (a) in the lower part sandstone covered with marly limestone debris; (b) in the upper part marly limestone covered with humus (mould).

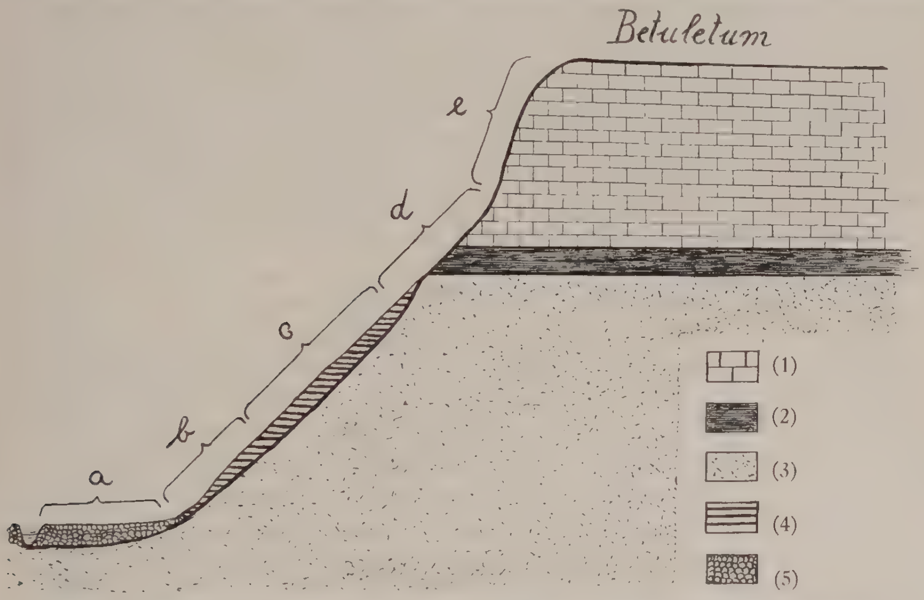


FIG. 3. Profile above the military shooting ground near Libušín (for explanation see text.) (1), Marly limestone; (2), Clay; (3), Sandstone; (4), Debris, marly limestone; (5), Soil.

IV. More open deciduous woods with an undergrowth of *Mercurialis*. The substratum is unleached marly limestone with a layer of very rich mould of considerable thickness.

V. Shaded slope with growths of *Aconitum vulparia*. Unleached marly limestone, substratum dry.

VI. Plateau with open birch wood, with Calluneto-vitis-idaeetum. The substratum is formed by washed-out marly limestones, and is very dry.

We see, then, that this zonal differentiation of plant associations in a limited locality is a classical example for the study of edaphic influences. In the mixed deciduous woods still occurs abundantly *Tilia platyphyllos*, which in the original forest was doubtlessly still more abundant. As a hard problem remain, of course,

the forest associations on washed-out marly limestones, where today we see for the most part dry and monotonous growths of *Pinus silvestris*, and in places also of *Betula alba* and *Picea excelsa*. The spruce, without doubt, is not indigenous here, but the pine probably is, though it seems that even in these places the original growth was a mixed forest, of course xerophilous, with pines and oaks predominating. To this the isolated stumps of aged oaks which we find in the pine forests bear witness.

11. The distribution of species according to substratum. The cretaceous marly limestones and sandstones represent in our district the two predominating types of substratum, which in their chemical and physical properties are absolutely different from each other, and the influence of which is felt even in the forest associations, for the unleached marly limestones promote the formation of mould or slightly acid, fine humus, while the sandstones and washed-out marly limestones create raw and acid humus. Some species in our district are confined to marly limestones for the reason that they are components of associations growing here exclusively on this substratum, while in other regions they grow in the same or even in other associations on a non-calcareous substratum. Some xerothermous species, as for example, *Dianthus Carthusianorum*, *Eryngium campestre*, *Poa bulbosa*, *Potentilla argentea*, and *Veronica prostrata* grow both on sandstones and on marly limestones. *Helianthemum obscurum*, however, gives marked preference to a calcareous substratum.

Of the species confined to a particular substratum I name the following:[4]

A. Species confined to marly limestone soils (unleached). *Aconitum vulparia!!*, very common and gregarious; *Anemone silvestris!*, very common; *Anthericum ramosum*, scattered; *Aquilegia vulgaris*, abundant; *Aster amellus*, only in places, very gregarious beyond Mayrau; *Brachypodium pinnatum*, often gregariously; *Brunella grandiflora*, only in places; *Bupleurum falcatum*, scattered; *Carex glauca*, common, especially on damper, argillaceous ground; *Carex humilis*, on the whole is not widely spread, not forming any special associations; *Caucalis daucoides!*, gregariously in fields, especially at the foot of the Vinarická hora, but also elsewhere; *Centaurea axillaris* (Triumfetti), in places in considerable abundance; *Cirsium acaule*, scattered, perhaps also crossing over on to sandstones; *Cirsium eriophorum!*, abundantly scattered; *Cirsium pannonicum!!*, very common; *Cephalanthera alba!*, rarely; *Clematis recta!*, scattered; *Conringia orientalis!*, only in places; *Coronilla vaginalis!!*, common; *Cotoneaster integerrima!*, not abundant, also on basalt; *Cypripedium calceolus!!*, on numerous, but of course scattered localities; *Dictamnus albus!!*, in Max's valley gregariously; *Festuca sulcata*, abundantly; *Galium silvaticum*, abundantly; *Hierochloe australis!*, locally; *Lilium martagon!*, abundantly; *Lithospermum officinale!!*, only locally; *Melittis melissophyllum!*, on the whole abundant; *Nonnea pulla!*, scattered; *Ononis spinosa*, scattered; *Polygala amara!!*, scattered; *Potentilla arenaria*, on the whole infrequent; *Reseda lutea!*, scattered; *Rubus saxatilis!*, very abundant; *Sorbus aria!*, abundantly scattered; *Stachys germanica!*, abundantly scattered;

[4] The exclamation points designate species rather strictly confined to this or that substratum.

Tetrazonolobus siliquosus!!, in places on argillaceous soil; *Teucrium chamaedrys*, in places; *Thymus praecox!!*, common; *Thymus Bayeri!*, in places; *Viola mirabilis*, scarce.

B. Species confined to non-calcareous substrata, sandstones, and leached-out marly limestones: *Antennaria dioica*, scattered; *Anthericum liliago*, very scattered; *Arnica montana!!*, scattered; *Calluna vulgaris!!*, commonly distributed; *Campanula rotundifolia*, scattered; *Carex pilulifera*, infrequent; *Chamaebuxus alpestris!!*, on the whole abundantly; *Corynephorus canescens!*, on sandy soil, scarce; *Deschampsia flexuosa!!*, very common; *Dianthus silvaticus!!*, only very scattered; *Festuca ovina!*, very common; *Genista germanica*, only very scattered; *Galium rotundifolium*, scattered; *Helichrysum arenarium!*, on sandy soil, infrequent; *Peucedanum oreoselinum!!*, scattered; *Platanthera bifolia*, infrequent; *Potentilla tormentilla*, very scattered; *Rumex acetosella!*, frequently; *Spergularia Morisonii!*, on sandy soil, scarce; *Thymus angustifolius!*, on sandy soil, locally; *Vaccinium myrtillus!*, very common; *V. vitis idaea!!*, commonly distributed; *Viola canina*, scattered.

C. Basalt species. On the basalt soil of Vinarickă hora grow some calcicolous species; and, besides, the following species are represented exclusively on it:

TABLE 6. *CALLUNETO—VITIS IDAEETUM*

	I					II				
	1. m²	2. m²	3. m²	4. m²	K %	1. m²	2. m²	3. m²	4. m²	K %
Vaccinium vitis idaea	1	1	1	1	100	1	1	1	1	100
Calluna vulgaris	1	1	1	1	100	1	1	1	1	100
Deschampsia flexuosa	1	1	1	1	100	1	1	1	1	100
Festuca ovina	1	1	1	1	100	1	1	1	1	100
Polytrichum piliferum	1	1	1	1	100	1	1	1	1	100
Luzula campestris	1	1	..	1	75	1	1	1	1	100
Hieracium murorum	..	..	..	..	...	..	..	..	..	...
Hieracium vulgatum	..	..	1	..	25	..	..	..	..	...
Carex montana	1	..	..	..	25	..	..	..	..	...
Carex pilulifera	..	..	..	..	...	..	1	..	..	25
Antennaria dioica	..	..	1	..	25	..	..	..	..	...
Galium silvestre	..	..	..	..	...	..	..	..	..	...
Myosotis stricta	..	..	..	..	...	..	..	..	..	...
Cerastium caespitosum	..	..	..	..	...	..	..	..	..	...
Quercus robur	..	..	..	..	...	..	1[a]	..	..	25
	III					IV				
Vaccinium vitis idaea	1	1	1	1	100	1	1	1	1	100
Calluna vulgaris	1	1	1	1	100	1	1	1	1	100
Deschampsia flexuosa	1	1	1	1	100	1	1	1	1	100
Festuca ovina	1	1	1	1	100	1	1	1	1	100
Polytrichum piliferum	1	1	1	1	100	1	1	1	1	100
Luzula campestris	1	1	1	1	100	..	1	1	1	75
Hieracium murorum	..	1	..	..	25	1	..	1	..	50
Hieracium vulgatum	..	..	..	..	...	..	..	..	..	...
Carex montana	..	..	..	..	...	..	..	..	..	...
Carex pilulifera	..	..	..	..	...	..	..	..	..	...
Antennaria dioica	..	1	..	..	25	1	..	..	..	25
Galium silvestre	..	..	1	..	25	..	..	..	..	...
Myosotis stricta	..	..	..	..	...	..	1	..	..	25
Cerastium caespitosum	..	..	..	..	...	..	..	..	1	25
Quercus robur	..	..	..	..	...	..	..	..	..	...

[a] Two-year old seedling.

Alyssum saxatile (f. *Arduini*); *Festuca vallesiaca*, gregariously; *Stipa Joannis*, very scarce; *Thymus Löwyanus* var. *stenophyllus*, on the whole abundant.

Finally it must be mentioned that sandstones, either when water with dissolved calcium carbonate flows into them or when they are covered with marly limestone soil, support the species proper for calcareous substrata.

12. The minimi-area of *Calluna vulgaris-Vaccinium vitis idaea* association. On the washed-out marly limestones on the plateau above the military school grounds, mentioned before, above a slope on the upper part of which *Aconitum vulparia* predominates, there grows a hercynian vegetation with very sharp boundaries, that is, a birch wood (with isolated oaks) in which, however, a continuous *Calluna-Vaccinium vitis idaea* growth is developing unhindered. I analyzed this association, which floristically and physiognomically is poor and monotonous, at 4 places, each of which was at a considerable distance from the others. At each place I chose an area of 4 meters square, analyzing each quadrat separately. The results are given in tables.

These tables show that constant species appear in all cases already in the first square meter, *Luzula campestris* alone being represented in the fourth table only later, that is, in the second and following quadrats. We see, therefore, that the area of 1 square meter is the minimal area for this simple association (just as it is for Nardetum). Of course, when we go over the whole expanse of this exclusive association, the number of species in the lowest and lower classes of constancy is augmented. The species mentioned above (nos. 1–6), of course, are repeated constantly, while within the limits of the whole expanse of this association I found besides *Platanthera bifolia*, singly; *Convallaria majalis*, infrequently in small groups; *Hypericum perforatum*, not abundantly; *Epilobium angustifolium*, not abundantly; and *Myosotis silvatica*, infrequently. In a further continuation in which, however, the character of the growth changes somewhat, also *Viola canina* appears very infrequently, as well as *Calamagrostis arundinacea*.

LAS REGIONES BOTANICO-GEOGRAFICAS DEL ESTADO DE OAXACA[1]

C. CONZATTI
Oaxaca de Juárez, México

Cortésmente invitado por el Sr. Lester W. Sharp, Secretario del Comité encargado de formular el Programa a que se sujetarán las labores del IV Congreso Botánico Internacional, que abrirá sus puertas en la Cornell University de Ithaca, Nueva York, el 16 de agosto del ano en curso, para asistir personalmente a aquel científico Certamen, o en su defecto para presentar en él algún trabajo alusivo, en la imposibilidad material de cumplimentar lo primero, como bien desearíalo, cábeme el alto honor de satisfacer ahora a lo segundo, enviando este modesto estudio acerca de "Las Regiones Botánico-Geográficas del Estado de Oaxaca" para que de merecer los honores de la admisión, pueda ser leído—en alguna de las sesiones de la referida Corporación.

Bien sé que el tema escogitado es con mucho muy superior—a mis escasas fuerzas, pero el legítimo deseo de corresponder en alguna forma a la generosa hospitalidad que siempre me ha dispensado Oaxaca, indújome a abordarlo a efecto de dar a conocer en el exterior los detalles más sobresalientes de su exuberante—Flora, y pagar de este modo, no fuera más que en mínima parte, la inmensa deuda de gratitud que con ella tengo contraída.

Situado en el Sur de la República, de la cual ocupa aproximadamente la 22 ava parte, el Estado de Oaxaca, tanto por su posición geográfica, como por la riqueza de su suelo, está llamado a un halagüeño porvenir. Así lo hacen suponer las opulentas montañas que lo recorren en todas direcciones; la envidiable posición geográfica que guarda, y la reconocida importancia de su extenso litoral.

Limítanlo al Oeste Guerrero, al Norte Puebla y Veracruz, al Este Chiapas, y al Sur el Océano Pacífico en una extensión no inferior a 500 kilómetros, desde el Arroyo Arenas, que establece el límite entre Chiapas y Juchitán por el Este, hasta el Río Ometepec que lo separa de Guerrero por el Oeste.

Una longitud análoga recorre también la arista geográfica que partiendo del Cerro de la Gineta al Este de Juchitán, se dirige primero de Oriente a Poniente hacia el Centro del Estado, para desviarse en seguida rumbo al Noroeste y salir al de Puebla, después de servir de límite entre los Distritos de Huajuápam y Coixtlahuaca.

Esta arista, que separa las corrientes de agua que se dirigen al Golfo, de las que tarde o temprano llegarán al Pacífico, sufre una marcada depresión en la

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 17, 1926. (Read in briefer form by J. A. B. Nolla.)

parte Istmica del Estado, para levantarse luego considerablemente en el resto de su largo recorrido.

La anchura del Estado, considerada de Norte a Sur, de las inmediaciones de la Estación Camalote, sobre el Ferrocarril de Córdoba al Istmo, punto más septentrional de Tuxtepec, a Puerto-Angel, situado en la extremidad más meridional del Estado, es algo menor y puede estimarse en 325 kilómetros de longitud considerada en línea recta.

La superficie territorial del mismo se ha calculado en 92.000 kilómetros cuadrados, susceptibles de ser repartidos en tres partes más o menos iguales, una correspondiente a los Bajos de la tierra caliente; otra a los Valles de la tierra templada, y la tercera restante a las Serranías de la tierra fría.

Sumamente intrincado se presenta en él el sistema orográfico, y por esto mismo su estudio acertado resulta en gran manera dificultoso. A pesar de ello, empero, puede establecerse como un hecho incontrovertible que la arista geográfica a que me he referifo más arriba, divide al Estado en dos grandes Vertientes, la del Norte o del Golfo, y la del Sur o del Pacífico.

Las principales corrientes a que da origen la primera, están formadas por los Ríos Tonto, Quiotepec, Tuxtepec, Puxmetacán, Jaltepec, Sarabia, Malatengo y Coatzacoalcos, y los de la segunda, por los Ríos Ostuta, Juchitán, Tehuantepec, Copalita, Tonameca, Cuixtla, Verde, Ometepec y Mixteco, este último en el plano del Noroeste, que también corresponde al Pacífico, pero que va a formar parte, como afluente principal, de la Cuenca del Balsas.

De un modo general puede establecerse asimismo, que la Vertiente del Golfo es mucho más húmeda que la Vertiente del Pacífico, lo cual quiere decir, en último análisis, que la vegetación es, si no sensiblemente distinta en una y otra, sí por lo menos mucho más lujuriosa en la primera que en la segunda.

Esto depende, sobre todo, de que la acción de los vientos alisios determina en aquélla una precipitación pluvial abundante y prolongada, en tanto que en ésta, por hallarse substraída a la influencia de los mismos alisios, sólo tiene cortos periodos de lluvias cenitales, que duran mientras soplan los vientos del Suroeste, y en consecuencia su cantidad de humedad es mucho menor.

Comparando las floras de ambas Vertientes entre sí, se ve también que los límites altitudinales de las zonas boscosas han bajado en las del Pacífico respecto de las del Golfo, pues mientras en éstas se encuentran Encinas y Coníferas sólo hasta 1200 y 1850 metros respectivamente, en aquéllas comienzan a hallarse desde 650 y 1000.

En vista de todo esto, pero principalmente en atención a los deficientísimos datos climatológicos, altimétricos y meteorológicos fehacientes, alusivos al Estado de Oaxaca, yo me he pregundado en más de una ocasión durante el tiempo que he invertido en la preparación de éste mal pergeñado estudio, si será empresa factible tratar de establecer una distribución científica de los vegetales en el extenso territorio oaxaqueño, sin contar con los indispensables elementos de exploración que acabo de mencionar, y la respuesta uniforme que he obtenido de mí mismo acerca del particular, ha sido y sigue siendo negativa.

Una empresa semejante sólo será posible cuando pueda apoyarse en observaciones prolongadas sobre la meteorología y climatología del Estado, de las que hoy se carece casi completamente, secundada por el conocimiento perfecto de las plantas que lo pueblan, y por tanto éste mi estudio sólo debe tomarse como un esforzado ensayo que acaso contribuya a llegar más tarde al resultado que señalo, mas nunca como una clasificación definitiva sobre la Geografía botánica de Oaxaca.

Y no podría ser de otro modo toda vez que los trabajos existentes acerca de la Geografía botánica de México se refieren de preferencia a regiones muy conocidas y bien exploradas, como lo son Córdoba, Orizaba, Jalapa, las Cumbres de Maltrata, los Valles y Nevados de la Mesa Central, el Pico de Orizaba, el Litoral del Golfo, y algo también el Istmo de Tehuantepec, pero sólo accidentalmente hablan de la distribución de los vegetales en el Estado de Oaxaca.

A este respecto yo he consultado con todo detenimiento los trabajos de los muy competentes naturalistas Martins y Galeotti, sobre la distribución de los Helechos Mexicanos; de Galeotti, alusivo a la distribución de las Orquídeas Mexicanas; de Grisebach, acerca de la vegetación del Dominio Mexicano; de Fournier, sobre las Regiones Botánicas de México, y de Hemsley, relativo a su Flora de México, y en ninguno de ellos me ha sido posible hacer entrar la distribución total de las plantas del Estado de Oaxaca.

Aquellos trabajos, notabilísimos bajo cualquier aspecto que se les examine, han sido elaborados preferentemente para las localidades a que me acabo de referir; Oaxaca no podía entrar allí sino en ínfima parte, porque numerosas regiones de su vasto territorio—Juchitán, Tehuantepec, Choapam, Putla, Juquila, etc.—distan mucho de haberse explorado como lo merecen, y hasta creo poder decir que la única porción regularmente explorada de su suelo es la faja de terreno existente entre los meridianos 2° y 3°, al Oriente de México, que pasa de Norte a Sur por el Centro del Estado, con una anchura aproximada de 100 kilómetros a uno y otro lado del Ferrocarril Mexicano del Sur, y a lo largo del camino que va de Oaxaca a Puerto Angel.

Fuera de ella pocas noticias se tienen de las plantas silvestres que habitan los Distritos del Este y del Oeste.

Tampoco puede entrar por entero la Flora oaxaqueña en los Cuadros de las Regiones botánico-geográficas de México, de los distinguidos Naturalistas mexicanos, Dr. José Ramírez y Prof. Alfonso Herrera, Sr., por su carácter demasiado general y por las causas antes aducidas, lo que no impide sin embargo que la vegetación del Estado se adapte en buena parte a esos Cuadros, y que para mí hayan constituído un inestimable guía en la preparación del presente ensayo.

Cosa análoga debo decir también de los estudios que he citado no ha mucho, pues estando hechos por celebridades en la materia no pueden menos que resultar una ayuda valiosísima para todo aquel que se aventura en asuntos de tan difícil apreciación—como son los relativos a la Geografía Botánica en general.

Y menos mal que los pocos datos meteorológicos, altimétricos y climatéricos que se tienen sobre el Estado de Oaxaca fueran siempre exactos; pero ni

siquiera con esa ventaja se cuenta. Consultadas a este respecto la "Memoria Administrativa de Oaxaca" por el estadista oaxaqueno Manuel Martínez Gracida, correspondiente al año de 1883, y la "Geografía Histórica del Estado de Oaxaca," año de 1913, por el profesor Cayetano Esteva, encontré datos tan contradictorios como los que aparecen en el Cuadro siguiente, alusivos a algunas alturas:

Cabeceras	*M. Gracida*	*Esteva*
Choapam	600	1460
Cuicatlán	125	603
Nochixtlán	1715	1898
Pochutla	850	163

y por este tenor todo lo demás.

En Meteorología los únicos datos que se tienen—que yo sepa cuando menos—refiérense a la Capital del Estado, y han sido tomados en el Observatorio Meteorológico de su Instituto, por el Dr. Agustín M. Domínguez. De una memoria publicada por dicho Observatorio, cuya fecha me es imposible citar, tomo los siguientes datos alusivos a la Ciudad de Oaxaca.

Altura absoluta sobre el nivel del mar. Mts.	1555
Temperatura media anual	20°214
Id. máxima absoluta	35°4
Id. mínima absoluta	3°0
Presión barométrica media	636.34
Ebullición del agua, término medio	95.15
Días lluviosos en el año	121
Milímetros de lluvia	844

En 1908 o 1909, no estoy bien seguro, se instaló en cada Cabecera de Distrito una Estación termo-pluvio-métrica a cargo del Prof. de la localidad, quien recibía por este servicio un sobresueldo a título de gratificación; pero a poco andar, por motivos que no son del caso, las Estaciones dejaron de funcionar.

De no haber sido por tan adversa circunstancia, Oaxaca contaría hoy con datos meteorológicos suficientes para proporcionar una idea bastante exacta de su climatología y permitir una metódica distribución geográfica de su exuberante Flora. No siendo así y habiendo aún muchas regiones insuficientemente exploradas en su vasto territorio, resulta que la Geografía botánica del Estado está todavía por hacerse, y todo lo que se pretenda realizar ahora en este terreno, por fuerza debe tener carácter provisional.

Por fortuna, en territorios esencialmente tropicales como lo es Oaxaca—todo el Estado se halla entre los paralelos—15°43′ y 18°25′ latitud norte—el clima está en razón directa de la altura del suelo sobre el mar, de tal modo que a lugares bajos, corresponde en ellos clima caliente, y a lugares altos, clima frío, favorable circunstancia que me ha permitido elaborar la presente clasificación climatológica del Estado, basada de preferencia en la altimetría del mismo, único elemento con el cual he podido contar en parte.

Permitidme, honorables Congresistas, que me detenga todavía un momento para citar algunas curiosas peculiaridades alusivas a Oaxaca.

En el territorio oaxaqueño tienen nacimiento los Ríos más caudalosos de la República; el Coatzacoalcos, en la Serranía de Juchitán; el Papaloápam, por

medio del Quiotepec y otros, en la Sierra de Ixtlán; el Mixteco, como afluente importante del Balsas, en la Mixteca Alta; el Verde, que arranca del corazón del Estado con el nombre de Atoyac, y lo recorre en una longitud aproximada de 300 kilómetros, en las vecindades de las Sedas.

También se encuentran aquí los monumentos arqueológicos—más grandiosos del país, o sean las celebradas Ruinas de Mitla, sobre cuyo camino los viajeros admiran asombrados el Ahuehuete más colosal del planeta, y los de Monte Albán.

La depresión más pronunciada de la gran Cordillera se halla en la parte occidental del Itsmo de Tehuantepec, y el Ferrocarril Nacional de este nombre sólo asciende 200 metros para salvarla.

El suelo de Oaxaca es calizo-esquitoso en la Vertiente del Golfo, y calizo-cristalino, gnéisico o granítico, en la del Pacífico.

Por último diré que cuando el benignísimo clima que se disfruta en esta encantadora porción del Estado que se llama Valle de Oaxaca, sea mejor conocido, muchos magnates que ahora viajan por economía o distracción vendrán a radicarse aquí. Experimentar la benignidad de este temperamento siempre igual, es quedarse en él.

El Cuadro fitogeográfico provisional que yo admito para el Estado de Oaxaca, es como sigue:

A-Región caliente De 0 a 1200 m.	Subregión caliente del Litoral. Subregión cal. de la Costa y Cañada de Cuicatlán. Subregión caliente del Bosque tropical.
B-Región templada De 1200 a 2000 m.	Subr. tem. inferior o de los Valles Centrales. Subr. templada superior o Mixteco-cuicateca.
C-Región fría De 2000 a 3400 m.	Subregión fría de las Serranías elevadas. Subregión fría de las Altas Cimas.

Dadas la extensión del territorio a que se refiere y la variadísima Flora que lo puebla por una parte, y la necesidad o por lo menos conveniencia de conciliar el aspecto científico del asunto, con la costumbre popular común y corriente que sin excepción considera dividido el Estado en tierra caliente, templada y fría por la otra, conforme al estilo del país, que es distinto del que impera respecto de estos mismos términos en Europa y América del Norte, excuso decir que la admisión del Cuadro que antecede ha sido motivo para mí de muy largas meditaciones, y puedo presentarlo como el resultado final de varios ensayos similares, ya que para su adopción definitiva tuve que luchar mucho conmigo mismo entre los que se disputaban mi preferencia.

Para elaborarlo me he apoyado principalmente, primero en las autorizadas sugestiones, pocas por desgracia, de los eminentes trabajos de que tengo hablado al principio de este estudio; secundariamente en los datos climatéricos que he podido reunir de aquí y de acullá, y por último, en el conocimiento personal que he adquirido del Estado en los treinta y cinco años que llevo de recorrerlo en distintas direcciones.

Apresúrome, sin embargo, a declarar luego que con ésto no quiero significar que yo haya visitado todo su territorio, por cuanto que son numerosísimas las

localidades del mismo que no he tenido oportunidad de ver nunca, sino tan sólo que he excursionado por él en rumbos diversos, siempre colectando plantas que ahora se encuentran depositadas en diferentes centros científicos—Universidad de Harvard, Instituto Smithsoniano de Washington, Dirección de Estudios Biológicos de México, y en mi Herbario particular, Así, pues, las plantas en que se basan las Subregiones de mi Clasificación, en su mayor parte han sido colectadas por mí.

Para que se pueda apreciar más fácilmente la índole de este trabajo, estimé conveniente acompañarlo de una Carta en la cual cada Subregión se distingue de las demás por un tinte diferente.

Con su auxilio pienso que será siempre sencillo darse cuenta de las localidades en que las plantas han sido colectadas.

Adelantándome a la crítica, debo decir que en rigor de la verdad, y según el criterio clásico en asuntos de esta naturaleza, Oaxaca tiene de hecho las tres Regiones climatéricas mencionadas, aunque no con la misma extensión que yo les señalo, para amoldarme a la costumbre popular imperante en el Estado. Para poner de acuerdo la Clasificación admitida por mí con el criterio clásico a que acabo de aludir, sería necesario refundir la Subregión que llamo templada superior o Mixteco-cuicateca, en la templada inferior o de los Valles Centrales, para que la Subregión de las Serranías elevadas de mi Región fría bajara con el mismo nombre a constituir la Subregión templada superior, de tal modo que en la Región fría sólo quedara la correspondiente a las Altas Cimas, con lo cual se obtendría el Cuadro climatérico siguiente:

Región caliente................. De 0 a 1500 m.	Subregión caliente del Litoral. Subr. cal. de la Costa y Cañada de Cuicatlán. Subregión caliente del Bosque tropical.
Región templada................ De 1500 a 3000 m.	Subr. temp. inferior o de los Valles Centrales. Subr. temp. superior o de las Serranías elevadas.
Región fría...................... De 3000 a 3400 m.	Permanece indivisa y comprende las Altas Cimas.

Como se vé, ésta nueva clasificación ofrece ciertamente la ventaja de estar más conforme con los principios científicos admitidos, pero en cambio tiene el inconveniente de hallarse en desacuerdo con el sentir popular que considera tierra fría la Subregión de las Serranías elevadas, la cual no abarca menos de un kilómetro en sentido vertical, y se encuentra comprendida entre los 2000 y 3000 metros de elevación, al mismo tiempo que intercalada entre la Subregión de los Valles Centrales y la Subregión Mixteco-cuicateca, separando una de otra poco menos que íntegramente. Esta intercalación, como puede verse en la Carta adjunta, constituye, de hecho, el carácter diferencial más importante entre las dos Subregiones de mi Región templada, y justifica con amplitud mi proceder.

Aquí, sin embargo, se impone una aclaración necesarísima de mi parte: las alturas de que me sirvo para delimitar las Regiones y Subregiones, no tienen, ni con mucho, la rigidez que a primera vista parece desprenderse de los números correspondientes.

En la zona intermediaria o templada, sobre todo en sus límites extremos inferior y superior, aquellas cifras deben interpretarse en sentido elástico o convencional, nunca con el carácter de absoluta inflexibilidad que tiene siempre la representación numérica de toda cantidad, porque aquí las Regiones se entremezclan de modo que sus mismos vegetales característicos pasan de una a otra con la mayor facilidad.

A este respecto precisa no olvidar que las regiones y subregiones de toda clasificación fitogeográfica, cualquiera que ella sea, se compenetran sin cesar, a tal grado que resultaría absurdo pretender trazar límites inflexibles de demarcación entre unas y otras. Y esto que es cierto aún tratándose de plantas locales o propias de determinada región, lo es todavía mucho más cuando se consideran otras que se adaptan indistintamente a dos o más regiones, habiéndolas que pueden recorrerlas todas sin mayores inconvenientes, como por fortuna sucede con algunas cultivables, verbigracia, el Frijol, la Patata y el Maíz.

Con estas salvedades, que me han parecido pertinentes, paso a reseñar los caracteres sobresalientes de cada Región y Subregión, no sin advertir antes que, en obvio de la brevedad, omito en ellas las largas listas de plantas en que se apoyan, y que yo colecté en las diferentes exploraciones botánicas que he logrado realizar por el extenso territorio oaxaqueño. Posible es que más tarde me resuelva a publicarlas, como que son el fundamento básico del presente ensayo.

Compónense dichas listas de numerosísimas especies pertenecientes a más de 150 familias de la entera escala vegetal, varias de ellas representadas en todas las Subregiones del Cuadro, como se observa respecto de las Polipodíaceas, Gramíneas, Leguminosas y Sinantéreas, que suelen encontrarse desde el litoral hasta el último picacho del Zempoaltepetl. Otras familias, sin tener tan grande extensión, recorren con todo varias zonas del Estado, tal como acontece con las Anacardiáceas, Malpighiáceas, Orquídeas, Enoteráceas y Borragináceas, habiéndolas por fín que no gustan salir de su región, como por ejemplo las Rizoforáceas, Fuquieráceas, Valerianáceas y otras más.

Así pues, me limitaré a citar en cada ocasión muy contadas especies, principalmente de las descubiertas en el primer cuarto del siglo actual.

.Las Regiones Botanico-Geograficas

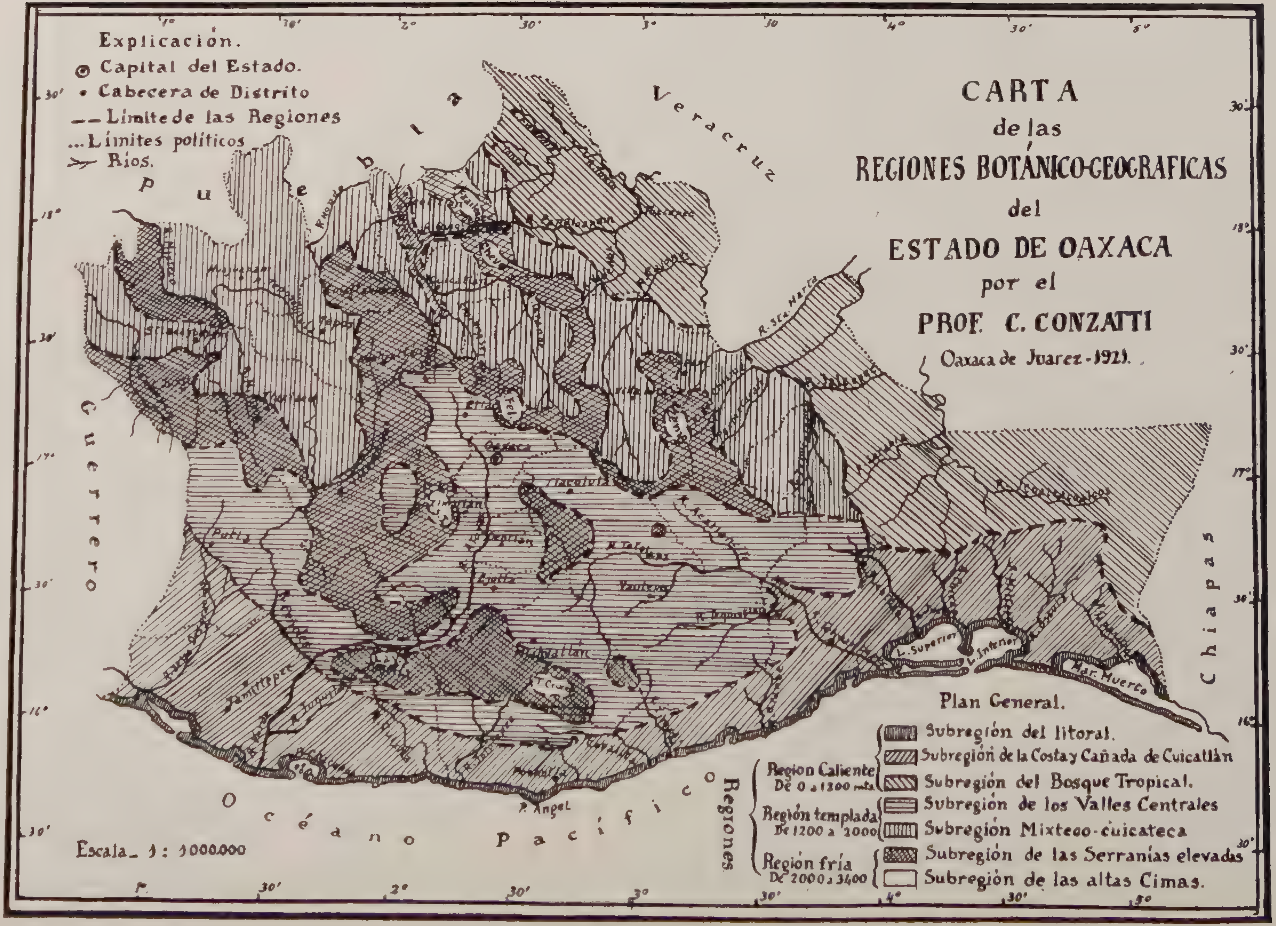
A—Region Caliente

En la Vertiente meridional esta Región suele ir de 0 a 1200 metros de elevación, y de 100 a 1500 en la Vertiente septentrional. Su temperatura media anual oscila entre 25° y 30° en los lugares bajos, pudiendo bajar hasta 20° o 19° en los más elevados.

Se divide en tres Subregiones bien marcadas, la segunda de ellas subdividida en tres Estaciones distintas:

I—SUBREGION CALIENTE DEL LITORAL

Es semi-húmeda y está formada por una faja angosta y continua de Jamiltepec a Juchitán, principalmente arenosa, paralela al Océano y expuesta directa-


CARTA
de las
REGIONES BOTÁNICO-GEOGRAFICAS
del
ESTADO DE OAXACA
por el
PROF. C. CONZATTI
Oaxaca de Juarez - 1921.
Explicación.
Capital del Estado.
Cabecera de Distrito
Límite de las Regiones
Límites políticos
Ríos.
Puebla
Veracruz
Guerrero
Chiapas
Océano Pacífico
Oaxaca
L. Superior
L. Inferior
Mar Muerto
Plan General.
Regiones
Region Caliente De 0 a 1200 mts
Región templada De 1200 a 2000
Región fría De 2000 a 3400
Subregión del litoral.
Subregión de la Costa y Cañada de Cuicatlán
Subregión del Bosque Tropical.
Subregión de los Valles Centrales
Subregión Mixteco-cuicateca
Subregión de las Serranías elevadas
Subregión de las altas Cimas.
Escala 1 : 3000.000

mente a la influencia del mismo. Su altitud puede calcularse en 100 metros, y como plantas características de sus diferentes parajes cuenta:

(a) En rocas sumergidas, varias algas, entre ellas una Feofícea y tres Rodofíceas.

(b) En lagunas y esteros, la curiosa planta tanante, *Rhizóphora mangle*, arbolito sociable de raíces aéreas entrelazadas de mil modos diversos, al grado de permitir andar sobre ellas, y el *Acróstichum excelsum*, grande helecho acuático del Charco de Minizo.

(c) En las arenas de la playa, las gramíneas *Anthéphora élegans*, *Bouteloua aristoides y Jouvea stramínea*, y la *Okenia hypogaea*, célebre Nictagínea que para madurar sus frutos introduce las flores fecundadas en la arena, al estilo del Cacahuate;

(d) En la playa y sus vecindades, la Majahua—*Hibiscus tiliáceus*—interesante malvácea por su corteza fibrosa.

(e) En las colinas del Litoral, varias Anonáceas, un Cactus y una pequeña Sinantérea—*Espejoa mexicana*—de aquenios grandes y canescentes.

Como se ve, las plantas arbóreas, que en el Litoral del Golfo casi no existen, tal vez debido a la violencia de los Nortes, en el del Pacífico no faltan, según aparece del contingente anterior.

II—SUBREGION CALIENTE DE LA COSTA Y CANADA DE CUICATLAN

Es seca de octubre a mayo, y húmeda de junio a septiembre. En algunos lugares sube hasta 1200 metros. En la Costa sigue inmediatamente a la anterior y como ella es contínua pero mucho más ancha. Es la Zona caliente seca de la Selva tropical. Su temperatura es elevada y en general parece árida, debido a la falta de lluvias durante dos terceras partes del año, pero en realidad es muy rica en especies vegetales, principalmente en las barrancas, sin duda por su proximidad al mar, y ésto lo tenía observado ya el ilustre Naturalista mexicano, Prof. Alfonso Herrera, padre, quien asienta que la vegetación en esta Zona es mucho más exuberante y variada que en la Costa oriental.

Abundan en ella las maderas finas y los árboles de gran talla, así como Leguminosas espinudas y frutescentes. También tiene diferentes formas de Cactáceas, pero sólo posee un Helecho arborescente y sus Orquídeas son escasas.

De manera provisional considero en ésta Subregión tres Estaciones principales, aunque por el momento me sea imposible caracterizar las dos primeras, en virtud de que sus especies, hasta donde puedo juzgar, son sensiblemente las mismas en ambas. Observaciones posteriores tal vez logren diferenciarlas más tarde.

a—Estacion Del Suroeste

Está formada por la porción costeña de los Distritos de Jamiltepec, Juquila y Pochutla. El Algodón y el Café son sus cultivos principales, por más que este último esté poco indicado. Abarca dos grados de longitud al oriente de México.

b—Estacion Del Sureste

Según se dijo ya es muy parecida a la anterior y como ella comprende dos grados de longitud al oriente del meridiano de México. La constituye la faja

costeña de los Distritos de San Carlos Yautepec, Tehuantepec y Juchitán. El cultivo del Añil es aquí uno de los principales.

Como especies características de la Costa oaxaqueña, cuyas—familias botánicas seguramente pasan de 50, son de mencionarse: el Cuapinole o Hymenaea Courbaril, árbol corpulento de buena madera; produce excelente resina para barníz; el Bálsamo o Toluífera pereirae, otro árbol grande muy apreciado; del Brasilito o Haematoxylon brasiletto, se exporta su madera por el tinte que proporciona; El Palo zongolica y el Ocotillo blanco—*Astronium Conzattii* Blake, y *Guarea Makrinii* Blake, respectivamente—ambos de Pochutla, son especies nuevas de madera muy fina; hay aquí también varios Pochotes todavía indeterminados, cuya lana sedosa nadie aprovecha, no obstante ser muy estimada; el Manzanillo o Hippómane mancinella, y la Cangura—*Rourea glabra*—son arbolitos justamente temidos por sus frutos venenosos; el *Diospyros Conzattii* Standley, por el contrario, es un nuevo frutal esquisitísimo del Cerro Espino, Pochutla, ahora cultivado en Oaxaca; citaré por último el Corozo o Attalea cohune, importante Palmera de frutos aceitosos.

c—Estacion Del Tomellin

Largo Cañón perteneciente a la Vertiente del Golfo, pero caliente y seco. Abarca dos Distritos—Cuicatlán y Teotitlán—que atraviesa de Sur a Norte en una longitud de 100 kilómetros, de Sta. Catarina a S. Antonio, con una altitud variable entre 550 y 1200 metros, sus flancos comprendidos. Su fondo sirve de lecho a los Ríos Salado y Quiotepec, que se juntan en el pueblo de este nombre. y ya reunidos se abren paso al través de la Sierra cuicateca que cortan de Poniente a Oriente para constituir luego el anchuroso Papaloapam.

La razón de la resequedad mencionada es que los Nortes del Golfo se disuelven al chocar contra la elevada Sierra cuicateca que corre paralela al Cañón por el Oriente y por lo mismo no ejercen influencia ninguna sobre esta Vertiente occidental interior de Cuicatlán y Teotitlán.

Esta Estación segúramente es muy distinta de las anteriores, pudiendo estimarse en más de 40 las familias fanerogámicas que la pueblan, y en ella abundan las Cactáceas, si no por el número de las especies, sí por el de los individuos. Carece de Orquídeas.

Sus principales cultivos son la Caña de azúcar y el Arroz. Como plantas connotadas de ella apuntaré las siguientes: La Giotilla—*Escontría chiotilla* Web. es un árbol hasta de 7 metros de alto, con numerosas ramificaciones verdes, costilludas y espinosas. Su fruto comestible alcanza cinco centímetros de diámetro. El Padre nuestro—*Myrtillocactus geométrizans*—es también árbol mediano con muchas ramificaciones hexagonales, sub-glaucas o verde-claras, y el frutito comestible que produce se denomina Garambuyo. La Tetetza—*Pachycéreus columna*—trajani—es un Céreo gigantesco, provisto de numerosas costillas longitudinales. Produce los llamados Higos de Tetetza. El Candelabro o Cardón del Sur—*Lemaireocéreus Wéberi*—es sin duda el ejemplar más hermoso y arrogante de todo el grupo: da un fruto grande y comestible. El Linaloé—*Elaphrium aloéxylon*—convertido en astillas—produce por destilación una

esencia muy estimada en perfumería. Aquí hay también un Pochote—*Ceiba aesculifolia*—que permanece inexplotado. Otra de sus plantas interesantes es el *Haplóphyton cimícidum* o Hierba de la cucaracha, por sus propiedades insecticidas. El *Exogonium Conzattii* (Greenm.) House, o Jícama del monte, de Almoloyas, ofrece bellas flores carmesí: sus raíces tuberosas son comestibles. En fin, el Chico-zapote—*Achras sapota*—se ha propagado mucho por los pueblos de la Cañada, a causa de sus excelentes frutos y de la óptima calidad de su madera.

III—SUBREGION CALIENTE DEL BOSQUE TROPICAL

Es muy húmeda, Corresponde a la Vertiente del Golfo y abarca todo el Distrito de Tuxtepec, noreste de Choapam, norte de Tehuantepec y parte de Juchitán en sus límetes con Veracruz y Chiapas. Por ser de difícil acceso, debido a lo mucho que llueve en ella y a su carencia de buenas vías de comunicación, está muy poco explorada, siendo bastante inciertos sus límites superiores, aunque estimo que pueden llegar en algunos lugares hasta 1500 metros.

Es muy rica en Palmeras para macetas, en maderas finas y en Helechos grandes y chicos. Sus bosques son impenetrables a no ser machete en mano, y no creo aventurado decir que pasan de 50 las familias botánicas que la pueblan.

Posible es que admita dos o tres Estaciones, pero yo no tengo datos para establecerlas. Con todo éxito se cultivan en ella el Cacao, el Hule, la Caña de Azúcar, el Tabaco, el Plátano y el Café.

Véanse algunas de sus connotadas plantas silvestres: El *Sphagnum meridense*, pequeña Muscínea de los bosques sombríos de Teutila, Cuicatlán, puede substituir al algodón absorbente. La *Alsóphila Schiedeana* es un hermoso Helecho arborescente de Tuxtepec. La *Sobralia macrantha* es una Orquídea terrestre de la Chinantla cuicateca, notabilísima por sus grandes flores color de rose. El *Dictyanthus tigrinus* Standley e Conzatti es una hermosa Asclepiadácea nueva de Ojitlán, Tuxtepec. El Hule o *Castilloa elástica* suele encontrarse al estado silvestre en los bosques de Tuxtepec, Choapam y norte de Tehuantepec. También el Achiote—*Bixa orellana*—es frecuente en los mismos lugares. De las cercanías de Ubero es una bonita Malvácea nueva—*Malache Conzattii*—así bautizada por el botánico Standley del Instituto Smithsoniano de Washington. Citaré por último el Yoloxóchitl—*Talauma mexicana*—del cual hay corpulentos ejemplares en Chiquihuitlán.

B—Region Templada

Va de 1200 a 2000 metros. Su temperatura media anual es de 20° o 19° en los lugares bajos, y de 16° o 15° en los elevados. Por su condición de intermediaria y la grande área superficial que abarca, es muy difícil de caracterizar. Está indicada para las grandes labores agrícolas y comprende dos Subregiones algo arbitrarias, tanto por lo que respecta a su escala vertical como a su grado de humedad, y acaso más acertado resultaría reunirlas en una sola, si entre ambas no se intercalase la Subregión de las Serranías elevadas que las separa completamente una de otra, determinando en ellas alguna diferenciación en cuanto a su grado de humedad.

I—SUBREGION TEMPLADA INFERIOR O DE LOS VALLES CENTRALES

Abarca de 1200 a 2000 metros. Ocupa la mayor parte del centro del Estado, entre los 16° y 17° de latitud norte, y comprende los Valles de Etla, Oaxaca, Zimatlán, Ocotlán, Ejutla, Tlacolula y norte de San Carlos Yautepec, con los flancos montañosos que los limitan. Es seca de noviembre a abril, y húmeda de mayo a octubre.

Sus principales cultivos son la Caña, el Trigo y el Maíz.

De entre las numerosísimas especies botánicas pertenecientes a más de 70 Familias que podría citar como propias de esta Subregión, me limitaré a presentar unas cuantas entresacadas de mi Herbario particular, para no hacer demasiado largo este trabajo:

CONZATTIA, Rose, género nuevo.

Contr. U. S. Nat. Herb. **12** (9): (see pp. 407–408. Pl. 59).

Fué establecido por el naturalista americano, Dr. J. N. Rose para una Leguminosa del Cerro San Antonio de la Cal, y admitido ahora como válido en el Suplemento del Index Kewensis. Actualmente comprende dos especies: *Conzattia arbórea* Rose, del lugar indicado, y *C. serícea* Standley, llamada Navío, de Mazatlán; *Ceiba acuminata* es un Pochote del Cerro Labrador, Ejutla; *Stauranthus Conzattii* Rose es una Rutácea nueva del Tule; *Fouquiera formosa* es un hermoso arbusto espinudo de las alturas de Matatlán.

Hicoria tetráptera, o Nuez de Cuilapam, es bastante común en todo el Valle.

El pueblo del Tule posee el Sabino más corpulento del orbe: su nombre botánico es *Taxodium mucronatum*.

En el Cerro de este mismo pueblo hay una Sapotácea nueva, *Bumelia eriocarpa* Greenman and Conzatti, posiblemente susceptible de cultivo.

Terminaré esta breve lista citando una azucena nueva del Fortín, Oaxaca—*Zephyranthes Conzattii* Greenman, cultivada hoy en muchas casas como planta ornamental.

II—SUBREGION TEMPLADA SUPERIOR O MIXTECO-CUICATECA

Es de muy difícil deslinde en su contacto directo con la—Subregión del Bosque tropical, donde ha sido poco explorada, y del cual es la continuación. Se extiende de 1500 metros o menos a 2000. La constituyen el Norte de Tlaxiaco, Teposcolula, Huajuapam, noreste de Coixtlahuaca, parte de Cuicatlán, Teotitlán, Villa Alta e Ixtlán. Se halla comprendida entre los 17 y los 18 grados de latitud septentrional, o sea un grado más al norte que la anterior Es muy húmeda en la Chinantla, algo húmeda en el resto de Villa Alta e Ixtlán, seca en las partes laterales de Tomellín y la Mixteca, excepto en la temporada de las lluvias. Creo que sin inconveniente serio podría incorporarse a la de los Valles Centrales de la cual propiamente no se diferencía sino por cierto grado de humedad, pero aún así habría que considerarla entonces como subdivisión de aquella.

La Caña y el Café se cultivan de preferencia en algunos lugares de la porción oriental, el Trigo en la occidental, y el Maíz en toda.

También aquí me sería fácil fundarla en una larga lista de plantas supeditadas a más de 30 familias, pero para el fín que persigo bastará con citar las siguientes: *Rhus toxicodendron*, de Cuyamecalco, muy empleado en medicina homeopática. *Euphorbia fulva*, de la Cuesta de Coyula: el látex de este árbol contiene un 20 por ciento de hule asociado a un 40 por ciento de resina, pero su separación resulta muy dificultosa. *Liquidámbar styracíflua*, de Cuyamecalco, es árbol interesante por el bálsamo que contiene. *Lamourouxia Conzattii* Greenman, de las Alturas de Pápalo, Cuicatlán, es una Escrofulariácea anual con bonitas flores ojas.

En esta Subregión, por fín, se encuentran también varias Orquídeas, entre ellas algunas Laelias, el *Arpophyllum gigánteum*, algunos *Epidendrum*, *Oncidium*, etc.

C—Region Fria

Se extiende de 2000 a 3400 metros de elevación, con una temperatura media anual de 15° o 16° en los sitios bajos, y 10° o menos en los más elevados. Conforme a las Clasificaciones más autorizadas, la Zona inferior de esta Región debería formar parte de la Templada, pero ya en otro lugar indiqué las razones que he tenido para considerarla como lo está aquí.

Se compone de las ramificaciones más elevadas del sistema orográfico oaxaqueño, el que por su naturaleza intrincadísima hace que esta Región sea en gran manera discontínua. En su mitad inferior todavía se cultivan con éxito los cereales, principalmente el Trigo y la Cebada.

Comprende dos Subregiones principales:

I—SUBREGION DE LAS SERRANIAS ELEVADAS

Es semi-húmeda y se extiende de 2000 a 3000 metros de altura. Se halla integrada por la Sierra Madre del Sur, Mixteca Alta, Sierras de Teotitlán, Cuicatlán, Ixtlán y Villa-Alta.

Entre sus plantas notables, correspondientes a más de 50 familias naturales, se cuentan: *Cheirostemon platanoides*, o Manita de león, de Pueblo Nuevo, Sierra Madre del Sur, y Yolotepec, Juquila, donde existe un bosque entero: tiene flores grandemente llamativas. *Aporocactus Conzattii* Britton and Rose es un Céreo de la Cumbre de Huehuetlán, susceptible de ser cultivado por lo vistoso de sus flores. *Drimys Winteri* o Palo de Chile, del Cerro La Raya, Cuicatlán, y Cumbre de Huehuetlán; proporciona la corteza picante de Winter.

Saurauja Conzattii Buscalioni, es árbol nuevo del Cerro La Raya, Cuicatlán. *Meliosma dentata*, de la Cumbre del San Felipe: la cáscara de su fruto contiene una materia colorante. *Quercus Conzattii* Trelease es una Encina nueva para la Ciencia, de la Cuesta de Huauclilla, Nochixtlán. *Gaultheria acuminata*, de las alturas de Pápalo, Cuicatlán, donde se conoce con el nombre de Arrayán, contiene esencia de Wintergreen, o Salicilato de Metilo, en la proporción de uno por ciento.

De diferentes localidades de esta misma Subregión, son también el *Abies religiosa*, el *Pinus oocarpa*, el *Juníperus fláccida*, y—otras mil más que no menciono por carecer de espacio para ello.

II—SUBREGION DE LAS ALTAS CIMAS

De 3000 a 3400 metros. Es semi-húmeda. Constituyen esta Subregión las Cimas que descuellan de 3000 metros, pertenecientes a las Serranías mencionadas en la anterior.

Tales son, entre otras, el Cerro Tres Cruces, en Miahuatlán; el Cerro de la Virgen, en Juquila; el Cerro de Santa Inés del Monte, en Zimatlán; la Cumbre de los Frailes, en Teotitlán; la Cumbre de Cheve, en Cuicatlán; el Cerro de San Felipe, en Ixtlán, y el núcleo del Zempoaltepetl, en Villa-Alta, que, según el geógrafo mexicano García Cubas, mide 4000 metros de elevación, y sólo 3400 según Horkart.

Como plantas características hay en ella las siguientes: *Callítriche verna Lomaria prócera, Cestrum nítidum, Gentiana adsurgens, Epidendrum élegans*, etc., Cerro de Santa Inés del Monte.

Polypodium Conzattii Weatherby, *Rhodosciadium glaucum lineare, Viola Pringlei, Arctostáphylos Conzattii, Abelia floribunda*, etc., Cumbre del San Felipe. *Dahlia variábilis* y *Aplopappus stoloníferus*, Cumbre de Cheve. *Deyeuxia triflora* (fide Liebmann) y *Fourcroea longeva* (fide Karwinski), Cerro del Zempoaltepetl.

Tal es a grandes rasgos la rica Flora de este Estado.

Seguramente que habrá mucho que corregir en la labor expuesta, pero en sus lineamientos sobresalientes tal vez pueda admitirse por el momento, a reserva que nuevos estudios y mejores observaciones del medio vengan a confirmarla o a substituirla con otra mejor.

Por más que en las diversas floras transcritas, correspondientes a las distintas zonas climatéricas de Oaxaca, sólo cité un mínimun de las numerosas plantas que las pueblan, claramente se colije por ellas cuán rica es la vegetación de este dominio, ya que en él se hallan representados los más grupos naturales de la República, siendo posible observar que a medida que se extiende la exploración científica por su extensa superficie, van apareciendo formas nuevas—Géneros y Especies—que no se hubiera pensado encontrar aquí.

Tal acontece, por ejemplo, con las plantas últimamente descubiertas, entre las cuales hay varios géneros que ameritan mencionarse.

Y así *SCHISMOCARPUS* (*S. páchypus*) es un nuevo género pochuteco de las Loasáceas; *OAXACANIA* (*O. malvaefolia*) es otro de las Sinantéreas encontrado en Almoloyas. *Bernoullia flammea, Erblichia odorata*, y *Rheedia édulis*, halladas en la flora silvestre de Pochutla, son formas esporádicas que se han escapado seguramente de la América Central, con otras más que me abstengo de citar para no ser difuso.

Algunas familias tienen su principal asiento en el Norte de México, pero esto no obsta para que se las encuentre también en Oaxaca y otros Estados del Sur; tal es el caso para las Lennoáceas, Orobancáceas y Monotropáceas, plantas singulares, diversamente coloreadas y sin hojas, parásitas sobre las raíces de otros vegetales.

Cierto número de plantas tienen aquí el carácter de naturalizadas o introducidas de otras partes. Es este el caso para el Arbol del pan, Artocarpus incisa,

de la tierra caliente húmeda; el Cocotero, Cocos nucífera; el Almendro costeño, Terminalia catappa; la Flor del Fuego, Poinciana regia, la Habilla de Guatemala, Hura crépitans; y la Ipomoea Quamoclit, de la tierra caliente seca; el Mango, Mangífera índica; el Níspero del Japón, Eriobotrya japónica; el Eucalipto, Eucalyptus glóbulos, y el Arbol del Perú, Schinus molle, que se hallan ampliamente difundidos por la Zona templada.

Detenerme por más tiempo en deducciones similares a las apuntadas, tanto equivaldría a abusar de la hidalga indulgencia que habeis tenido al impartirme vuestra atención por el tiempo que duró la lectura de este trabajo, y por ésto me es honroso presentaros los sentimientos de mi sincera gratitud.

SUR LA MÉTHODE ET LA NOMENCLATURE EMPLOYÉES DANS MON ETUDE GÉOBOTANIQUE DE L'ESPAGNE[1]

EMILIO HUGUET DEL VILLAR
Madrid, Spain

Dans la science comme dans les beaux arts, multitude d'écoles accuse exubérance de vie. Abstraction faite de la part d'erreur due à la limitation humaine, deux facteurs sont spécialement à signaler parmi les causes produisant la diversité d'écoles. L'un c'est l'idiosyncrasie de chaque investigateur. De ce point de vue, chaque méthode géobotanique est une façon de voir et d'exprimer le paysage végétal; tout-à-fait comme le style dans la peinture. Science et art répondent, sous forme différente, à une necessité du même ordre: de l'ordre esthétique. C'est aussi par sa beauté que la science nous attire. Dans celle du paysage végétal, pourvu que la vision soit vraie et l'expression juste, chaque méthode a son droit à la vie; et, dans le programme idéal de notre belle science ne doit pas figurer l'imposition d'une méthode unique à tous les géobotanistes, de même qu'il ne saurait pas être un idéal dans la peinture la soumission de tous les peintres à un style unique.

L'autre facteur ce sont les différences géographiques du paysage végétal lui même; chacun attirant l'attention sur un problème plutôt que sur un autre, ou offrant pour les même problèmes des différences d'aspect. Ainsi l'extension géographique de l'investigation peut bien contribuer à éclairer les problèmes de la méthodique. C'est l'interêt que peut avoir ce travail, l'Espagne étant un pays, d'une part très peu étudié encore avec des méthodes géobotaniques modernes, et d'autre part très intéressant par sa végétation.

À cause de la limitation inhérente à cette sorte de travaux, je dois me borner aux traits les plus généraux de la méthode que j'ai été porté à suivre, et, dans ceux-ci, seulement aux points offrant quelque nouveauté ou touchant à des questions très débattues.

Après la vérité et la justesse d'expression, il y a, à mon avis, un autre principe d'ordre général à observer dans la science: celui de la conservation des valeurs historiques. Il ne s'agit pas du simple droit de priorité que, depuis longtemps, les géobotanistes ont jugé n'être pas applicable ici comme il s'applique en Phytographie. En grâce de la conservation des valeurs historiques: (1) Une fois qu'un nom a été introduit avec un sens dans la science, il ne doit pas être appliqué à une notion différente: des idées nouvelles exigent des noms nouveaux. (2) Quand il s'agit de définir, le problème ne consiste pas à chercher ce que la chose doit être, mais ce qu'elle est réellement: non ce qu'un nom, depuis longtemps utilisé, doit exprimer, mais ce qu'on a voulu exprimer avec lui.

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 17, 1926.

Pour ce qui dans la science est susceptible d'être universalisé, il est à désirer une expression universelle. Et, puisque la Botanique a eu le bon sens de conserver le latin, je proposerais, comme principe additionnel, l'usage en Géobotanique d'expressions latines, comme on fait en Phytographie.

Les Unités Générales

Quoique en matière de successionisme j'ai suivi surtout les enseignements de Clements, le principe de conservation des valeurs historiques m'a empêché d'accepter, pour les mots formation et association le sens spécial que leur a donné ce savant, ainsi que pour formation celui créé par Moss et défendu en 1910 à Bruxelles pas le Comité Anglais. Si, pour une notion plus élargie nous continuons à employer le même nom, quel mot employer pour exprimer la vieille notion restrainte, qui doit subsister au moins dans l'histoire de la science?

Le mot formation fut légitimement créé par Grisebach en 1838 dans un sens clairement physiognomique, comme transformation plus spécialisée de celui de forme (Vegetationsform) employé en 1824 par Martius dans un ouvrage dont le titre commence par "La Physiognomie . . . ." et après lui par Lund et d'autres. Ce mot garde le juste parallélisme étymologique avec celui de "Hauptformen" employé par Humboldt en 1806 dans ses "Ideen zu einer Physiognomik der Gewächse." Ce ne fut qu'un quart de siècle après la création de Grisebach, que Kerner employa le même mot dans un sens équivalent à celui d'association collective. Depuis lors il a chancelé entre le sens originaire, l'écologique, le floristique et le successioniste. La définition proposée par les rapporteurs du Congrès de Bruxelles est encore un compromis entre les trois premiers penchants, et n'arriva quand même à obtenir qu'une majorité dans la Commission. Mais depuis lors un usage de plus en plus généralisé, dans lequel comptent l'école d'Upsal et la nomenclature Braun-Pavillard, tend à restituer au nom son sens primitif. Il y a donc trois raisons—l'étymologie, l'origine historique, et le penchant de l'opinion actuelle—pour que le mot formation reste à jamais fixé dans son sens purement physiognomique.

Il arrive de même pour le mot assotiation, créé par Humboldt en 1805 en vue de la composition floristique, purifié et à nouveaux sacré par Flahault au Congrès de Paris de 1900, et couronné aujourd'hui du consensus universel.

Mais j'ai éprouvé aussi le besoin d'un mot plus général, embrassant les deux notions, et exprimant à lui seul l'unité synécologique ou phytosociologique suprème et absolue, dégagée de tout point de vue spécifiant. En anglais et dans les langues néolatines on emploie pour cette idée une composition de deux mots au moins, en Allemand des mots composés différents dans chaque auteur. J'ai proposé à l'usage universel le mot gréco-latin synoecia (en français synécie), dont l'étymologie s'accorde exactement avec ce que l'on veut exprimer: la cohabitation. Ce mot ferait pendant à symbiosis, qui exprime la convivence.

Je ne sais pas si le rigorisme de l'école d'Upsal (pour lequel je professe de la vénération) ira jusqu'à trouver ce mot contaminé de parti pris localiste. Aux blâmes possibles je réponds d'avance que la deuxième partie du mot ne cache aucun guet-apens stationnel. Je dérive synoecia de et, habiter; et c'est évident

surtoutes les écoles, que les plantes dont il s'agit cohabitent dans un lieu, quel que ce soit. Je suis d'accord avec les enseignements d'Upsal sur ce que la méthode doit être inductive, aller du connu à l'inconnu, du groupement des plantes à la recherche du milieu. Mais justement, du groupement des plantes, c'est la cohabitation ce que nous voyons directement et tout d'abord. Les "rapports mutuels des plantes qui cohabitent" sont une notion discursive, un objet à investiguer. L'intuition nous fait savoir seulement que les plantes cohabitent. Il est à rechercher après si cette cohabitation est due aux rapports des plantes entres elles ou au rapport de chacune avec le même milieu, ou quelle partie correspond à chaque phénomène. Ce doit donc être la cohabitation, et non le fait sociologique, le "cogito ergo sum" de la méthode inductive. Le fait sociologique comme point de départ est un parti pris, non moins blâmable, que celui du rapport écologique objectif, si blâmé par les upsaliens. Les deux ont le même titre à la qualification d'hypothèse.

Ces considérations touchent déjà au problème de la dénomination générale de notre science, que pour ne pas enchevêtrer les questions, je traite plus loin.

La notion de synécie permet de donner, pour les grandes unités synécologiques, des définitions très simples, à la fois d'accord avec les valeurs historiques et l'étymologie.

Synécie c'est une cohabitation botanique individualisée.

Individualisée veut dire: unifiée (par la communauté de caractères en dedans de sa propre étendue) et délimitée (c'est à dire, distincte par eux mêmes des autres cohabitations). Dans tout ça il n'y a rien de préjugé, et on ne sort pas des termes les plus généraux. Ce sera le facteur individualisant que nous voudrons prendre en considération dans chaque cas, qui nous menera aux unités spécifiées.

Formation c'est la synécie du point de vue biomorphologique. (C'est à dire: une cohabitation botanique individualisée par la forme biologique qui y domine.)

Association c'est la synécie du point de vue systématique. (C'est à dire: une cohabitation botanique individualisée par sa composition floristique.)

On n'a pas besoin d'ajouter rien de plus. Le parti pris du rapport écologique reste exclu, non moins que celui du rapport sociologique. Et aussi toute hautise de subordination. Si beaucoup de géobotanistes ont cru devoir subordonner l'assotiation à la formation, c'est dû à ce que les formes biologiques que l'homme distingue, étant moins nombreuses que les espèces qu'il a admirées, beaucoup d'espèces lui apparaissent sous la même forme, et le nombre de formes dominantes qui résulte beaucoup moindre que celui d'espèces dominantes, et en conséquence le nombre d'associations plus grand que celui de formations. Mais il y a des cas où il arrive le contraire. Ainsi en Espagne l'association de *Quercus ilex* (la plus éxtendue dans l'aire xérophytique) peut se présenter sous deux formations: celle d'arboretum et celle de fruticetum, auxquelles le vulgaire donne même des noms différents.[2] La *Quercus coccifera* ne forme (à ce que je sache) en Espagne que des fruticeta, quand elle devient dominante; pourtant en Barbarie elle est aussi connue comme arbreplus ou moins élevé.

[2] Par exemple: *encinar* pour l'arboretum; *mata parda* (jamais "encinar" pour le fruticetum.)

Je sui donc d'accord avec Negri et avec Pavillard sur ce que la formation n'est que la physiognomie de l'association, et la formation est à l'association comme le biotype à l'espèce.

La Dénomination de Nötre Science

Presque toutes les sciences ont un nom, très souvent incorrect, mais dans la practique admis par tous. La nôtre n'en a pas, ce qui ne manque pas de conséquences facheuses. C'est une des innombrables difficultés auxquelles je me suis heurté dans mes efforts pour introduire en Espagne la Géobotanique moderne et y interesser les pouvoirs publiques. Plus d'une fois, quand j'ai parlé de Géobotanique, d'Ecologie ou de Sociologie végétale, on s'est mis à rire, en me demandant qu'est ce que c'étaient ces "choses si drôles," sans se douter du rapport qu'elles pouvaient avoir avec une autre certaine chose qu'on appellait Géographie Botanique et de laquelle on avait déjà quelqu' idée, quoique non tout-à-fait juste.

La dénomination de Géographie Botanique ou Phytogéographie employée en sens général, n'est qu'un phénomène de survivance. Elle ne convient en propre qu'à une partie de l'ensemble: celle des rélations avec le milieu géographique, qui ne peuvent être confondues avec les rapports avec le milieu stationel.

La dénomination de Phytosociologie, même en admettant que les problèmes auxquels elle s'applique soient en rèalité et exclusivement des problèmes sociologique (ce qui ménerait à admettre aussi la Géographie Humaine comme une partie de la Sociologie), ne peut non plus embrasser le tout, puisqu'elle exclue les rapports de chaque individu avec le milieu.

Je sais bien qu'il se pose ici une question non seulement de nom mais de fond, les sociologistes, comme DuRietz, font de ce qu'il s'agisse d'un organisme ou d'un groupement, le fondement de sa division biologique de premier ordre. C'est une division légitime, répondant à un tempérament déterminé, et qui contribue autant ou plus que d'autres, faites d'autres points de vue, à l'éclaircissement du système scientifique. Mais, puisque la vie végétale individuelle ou collective se développe toujours dans un milieu terrestre, rien ne nous empêche non plus d'envisager une discipline scientifique embrassant tous les faits de rapport avec le milieu, ou, si l'on veut pousser à l'extrême l'élimination d'idées préconçues, une science partant de l'habitat, et lui donner un nom (celui de Géobotanique par example), en la mettant en pendant avec l'étude qui s'attaque au fait biologique envisagé en soi même. Dans la discipline partant de l'habitat n'entrerait certainement pas le problème des formes physiognomiques, que cette branche doit emprunter à la branche biologique (stricto sensu) comme elle lui emprunte aussi les unités florales. Mais c'est tout autrement pour les synécies; puisque, comme nous venons de le voir, c'est la cohabitation et non les rapports sociaux des plantes entre elles, ce que nous connaissons directement et que nous devons prendre comme point de départ dans notre étude inductive. Et la cohabitation est déjà elle même un rapport avec le milieu: non de connexion car elle n'exprime rien de préalable sur l'influence de chaque facteur écologique); mais de situation.

C'est par l'étude de la cohabitation, que les sociologistes débutent eux mêmes. Ce qu'on se plait à appeller l'étude du groupement, de "son organisation," n'est pas autre chose. L'abondance, la surface occupée, la fréquence, la constance (soit dan le sens des upsaliens, soit dans celui de l'école de Zürich), l'aire minime, et même ce qu'on décore de qualifications psychologiques, comme la sociabilité et la fidélité, ne sont en somme que des relations quantitatives par rapport au milieu: des expressions quantitatives de la cohabitation.

De même pour la composition physiognomique ou floristique. Composition veut dire position ensemble, c'est à dire, situation dans une même unité locale. La qualification physiognomique de chaque élément ne se fait pas dans l'étude du groupement: un emprunt déjà fait à l'autre branche de la science, de même que la classification floristique: ici on se borne à constater la présence et proportion de ces éléments physiognomiques et floristiques dans une aire déterminée: toujours des aspects de la cohabitation.

Le "groupement en soi même," c'est à dire, les rapports biologiques des plantes entre elles (en tant qu'il y en a), c'est une toute autre chose: un objet à rechercher moyennant l'induction, non moins que les rapports objectifs, de connexion, entre les plantes et les facteurs du milieu. Et il faut bien avouer que, même en retranchant de se qu'on a cru savoir sur ces rapports écologiques de la plante, tout ce qui il puisse y avoir d'illusion et de parti pris, il y reste encore quelque chose de plus que ce qu'on connaît sur les rapports directs des plantes entre elles, outre ce qu'on a nommé symbiose.

Ces considérations éclairent, à mon avis, le sujet de la querelle entre écologistes et sociologistes, dans laquelle chacun a, comme il arrive souvent dans les querelles, sa partie de raison. Il faut distinguer entre les rapports de situation ensemble dans le milieu (ou cohabitation), et ceux de connexion avec les facteurs de la station (qu'on peut appeller facteurs écologiques, pourvu de s'en tenir ferme à ce sens concret du mot). Les sociologistes ont a mon avis raison, de nier que ces derniers rapports puissent être pris comme point de départ, et à affirmer qu' on ne les pourra saisir en tout cas que comme résultat de la recherche inductive. Mais si, par étude du groupement, mis à la base et commencement de cette induction, ils prétendraient entendre, non l'ètude de la cohabitation, mais celui des rapports directs entre les plantes, lesquels ne sont pas saisissables directement, ils tomberaient dans une erreur non moins grave que celle qu'ils ont devoilée et combattue. Et les écologistes auront par contre raison, si, en donnant au mot "écologie" le sens plus large de "rapport (quel que ce soit) avec le milieu," ils se tiennent, comme base de l'ètude, au simple rapport de cohabitation.

Ces considérations montrent que l'étude inductive des groupements végétaux peut bien rentrer dans une science de l'habitat, par laquelle le nom de Phytosociologie serait, non seulement trop restreint, mais même pas tout-à-fait accomodé. Si, de la recherche inductive commencée par la cohabitation, on arrive à la découverte de rapports directs des plantes entre elles mêmes, ces rapports auront aussi sa place dans la branche purement biologique, comme il

arrive pour la symbiose. Ce sera un apport de plus à controler, comme il y en a dans le sens inverse.

Le fait d'habitat et le fait biologique ne sont que deux portes différentes qui mennent au même tout, et les deux courants de visiteurs doivent se recontrer à l'intérieur plus d'une fois. Il arrive de même dans chaque groupe de sciences et dans tout l'ensemble scientifique. L'objet de la science est indivisible dans la nature. Les divisions dans l'étude humaine ne tiennent qu'aux différents points de vue, et, en conséquence, de départ. Je crois donc légitime dans la Botanique, ou Science du monde végétal, une division de premier ordre en Biobotanique, qui envisage comme point de départ le fait biologique, et Géobotanique qui envisage les faits d'habitat. Cette dernière comporterait, comme première subdivision, une partie idiologique, pour l'habitat des unités botaniques, et une partie synécologique, pour les synécies.

La division sociologiste en Idiobiologie ou étude des organismes isolement ou isolés ("Einzelorganismen" dans le tableau de DuRietz) et Biosociologie ou étude des groupements d'organismes ("Organismengesellschaften"), est non moins légitime et à la fois non plus tranchante; car les entités auxquelles on aboutit parfois dans l'étude idiobiologique—comme les genres, les familles et d'autres unités florales ainsi que les séries philogénétiques—ne sont pas moins des collectivités que les synécies et même avec des liens directs bien plus évidents.[3]

Le nom de Géobotanique, que la vogue du traité de Méthodique de Rübel a tant étendu dans ces dernieres années, a émoré un autre compétiteur, celui d'Ecologie, en faveur dans les pays de langue anglaise, et stéréotypé dans le titre des revues de la spécialité.

L'inconvénient que je trouve à ce mot, c'est la pluralité de ses acceptions. Comme dénomination de compartiment scientifique il en a été employé en trois: science du milieu (no. 1 de la proposition Jaccard au Congrès de Bruxelles); science des rapports des plantes avec la station (proposition 8 b de Flahault et Schröter dans leur Rapport au même Congrès); et science de l'habitat dans son sens le plus général.—Pour lui donner cette dernière acception, il faudrait dériver le mot, non de oinos, qui se rapporte à la station; mais de oinew habiter. Mais alors il faudrait renoncer à appeller facteurs écologiques au facteurs qui constituent la station, ce qui exigerait bien des corrections dans les textes scientifiques.

C'est pour ça que j'ai fini par octroyer ma préférence au nom de Géobotanique, créé et maintenu dans un seul sens. Mais il serait à désirer là-dessus un accord définitif.

La Succession et la Climax

J'ai commencé l'étude géobotanique de l'Espagne sèche sans aucun parti pris théorétique, ou, en tout cas, sous l'influence du sens écologiste prédominant

[3] Je traduis Gesellschaft par groupement, parceque c'est ainsi que le traduisent Braun et Pavillard dan son "Vocabulaire . . . ." qu'ils intitulent ". . . . de Sociologie Végétale." Du reste traduire le mot par *société* serait présupposer entre les végétaux aggroupés un lieu, que c'est justement ce qu'on doit démontrer. Malgré la dénomination générale admise, la cohabitation est souvent une lutte.

dans l'Europe continentale à l'époque de mes débuts. Des grandes étendues qualifiées de "steppes" par mes devanciers, je les envisageais comme de telles steppes, au sens dans lequel on donnait à ce mot; et ce fut justement cette étude, qui me mena à voir les choses tout autrement.

D'une part, dans ces prétendues steppes on réunissait des types de végétation fort différents; par exemple, des synécies ligneuses et sous ligneuses, comme les associations de *Quercus coccifera*, de *Retama sphaerocarpa*, de *Cistus ladaniferus* de Thymus, etc., sur des sols géognostiquement divers, mais toujours physiquement secs, en général plus ou moins ondulés, et pour la plupart pierreux; et des synécies presque exclusivement herbacées, abondantes en formes crasses et espèces franchement halophiles (quénopodiacées, plumbaginacées, etc.), en rapport avec le sol en dépression, humide et fortement salé—1, 2, 3% et plus de chlorures par exemple; pendant que dans les sols pierreux à végétation buissonante ou à tomillar on n'en trouvait que quelques dizaines, ou même moins de deux centaines par mil. La végétation ligneuse et sousligneuse était la plus étendue, la régionale, au centre, à l'est et dans le midi; elle débordait largement les limites, bien peu comordantes, assignées à la steppe centrale, par exemple, par les différents auteurs, et se confondait, tout simplement, avec la végétation générale, climatique, si l'on me permet pour une fois ce mot qui dans ce cas est le produit de l'expérience inductive. Les éléments floristiques de cette végétation ligneuse et sousligneuse formaient également, dans les clairières et le sousbois des taches éparses mais recurrentes du bois sclérophylle de *Quercus ilex* et de *Q. faginea*, dans l'est et le sudest aussi de *Pinus halepensis*, etc., que s'eparpillent par tout le pays, et qui paraissent même dessinées, quoique pas toujours assez exactement, sur les feuilles publiées de la Carte Topographique. De telles mouchetures d'arboretum osaient même se montrer en dedans de l'aire qualifiée de steppaire. Et aux taches de bois venaient se joindre, dedans et dehors, des arbres isolés des mêmes espèces éminemment sociales, comme des témoins éloquents d'un même passé. Même des graminées, comme celles des genres *Stupa* et *Macrochloa*, parfois réunies en groupements à faire penser à des véritables steppes originaires, dans le sens le plus restreint du mot, réapparaissaient aussi associées aux espèces ligneuses régionales dans les clairières et le sousbois.

Tout ça m'invitait à l'observation directe du fait plus que soupçonné. Malheureusement l'œuvre de déforestation se continnue en Espagne sous nos yeux avec l'activité la plus brutale. Les gouvernements ont observé toujours une attitude protectrice à l'égard de la cupidité du propriétaire qui, pour profiter d'une chance d'acquisition, d'un renchérissement du bois, ou des hauts prix du blé, artificieusement maintenus par le cercle douanier contre les besoins de l'alimentation publique, détruit d'un coup ce que la nature avait accumulé pendant des siècles, et aprés avoir profité des premières récoltes obtenues sur le sol créé par le bois, une fois qu'il commence à s'appauvrir et l'érosion à mettre à nu la roche du sous-sol, l'abandonne à un pâturage primitif, pour aller porter le ravage plus loin. Les documents abondent sur la déforestation depuis les commencements du XIX siècle. On en a aussi pour des époques plus reculées. Mais, sans avoir recours à l'histoire, on a assez d'occasions de voir le procès

par soi même, et de reconnaitre quelle végétation succède au bois détruit, comment elle recommence sur le champ abandonné, et comment elle se comporte à l'égard de l'exploitation abusive. J'en ai donc des raisons bien solides pour établir un rapport entre ces fruticeta et suffruticeta qu'on califiait de steppaires, et le bois sclérophylle que des témoins, dont plusieurs tombant l'un après l'autre sous mes yeux me montraient en récurrence à travers tout l'ensemble de l'Ibérie xérophytique, et pour en séparer les enclaves vraiment salines et locales.

Au même temps que le procès qui succède à la destruction du bois, dans le même paysage sec, ligneux, général, quand il s'agissait de sols sédentaires, comme sur les calcaires, les marnes magnésifères et les gypses du miocène, les granits et les gneiss des "sierras," etc., j'avais lieu d'observer un nombre de synécies clairement sériables, que, depuis les lichens entamant la pierre, et les mousses et en passant par des types de végétation fissuricole, comme le *Lithospermum fruticosum*, abutissaient aux même broussailles et buissons de la végétation ligneuse générale, quelques uns fissuricoles eux mêmes, comme la *Quercus coccif-erá*, et même au bois, dont des dominantes comme la *Pinus halepensis* ne refusent pas de croître elles mêmes dans les fentes des rochers.

Je fais grâce d'analogues considérations à propos de "vegas," montagnes, etc. Je me suis permis un peu de littérature pour montrer comment ce n'a pas été par parti pris, mais bien comme résultat d'un travail inductif, que j'ai dû chercher dans le doctrine et le vocabulaire successionistes de l'école anglo-américaine, la méthode que demandait le paysage végétal de l'Espagne.

On a fait à la méthode successioniste deux reproches. Le premier celui d'exclusivisme; la succession, a-t-on dit, n'est qu'une chose parmi tant d'autres à rechercher dans les groupements végétaux; ce n'est pas juste de la prendre comme point de vue. A ceci je réponds que la végétation n'est pas un phénomène statique, mais dynamique, et si l'on ne l'envisage et ne la décrit pas comme un mouvement, on s'eloigne artificieusement de la réalité. En Espagne, hors du point de vue successioniste, la végétation n'a pas de sens. Et il arrive de même d'une façon spéciale dans tous les pays où le paysage est très défiguré par la dévastation humaine. La région méditerranéenne est pour la plupart dans ce cas, une même cause phytogéographique ayant collaboré en dedans de ces limites avec l'avidité humaine: la lenteur de croissance de la végétation ligneuse xérophytique, ce qui veut dire une production de bois et de terre végétale trop inferieure aux besoins croissants de la population.

Le deuxième reproche est que la climax est une simple hypothèse, nature écologiste, pas toujours confirmée par l'expérience. Dans mon "Avancement sur la pretendue Steppe Centrale de l'Espagne" j'emploie aussi quelquefois le langage habituel des successionistes en rapportant la climax aux conditions du milieu, surtout au climat. Mais j'ai entrepris après la tâche de soumettre la notion de climax a une épreuve de purification, en démontrant qu'elle peut quand même subsister indépendamment de tout appui écologique. Elle n'a non plus besoin d'être éclaircie par une identification avec l'idée de "groupement final" (Schlussverein ou Schlussgesellschaft), qui est éliminable. Pourquois *final*? Avec quel droit coupons nous la nature? Si la végétation est une évolution,

nos Schlussvereine continueron à évoluer après nous, comme tous ont évolué des stades des temps passés qui auraient subit la même qualification s'il y avait en alors de géobotanistes. En tout cas la qualité de "final" n'aurait qu'une valeur relative, comme rapport à un état géophysique du milieu, lui même stade d'une évolution dans laquelle il serait aussi arbitraire de faire des coupures; et c'est justement aussi du rapport au milieu qu'il s'agit d'indépendeser originairement la notion.

Je crois qu'on y arrive en partant exclusivement de la synécie.

La série c'est une notion philosophique général, qu'on peut définir: ure somme d'entités en rapport antéro-postérieur. Quand ces entités sont des synécies, nous avons une série synècique. (On sous entend phytosynécique). Dire que chaque synécie de la concaténation est en double rapport avec l'antérieure et avec la suivante, ne préjuge rien sur la nature possible de ce rapport. Les causes déterminantes de la synécie (quelles qu'elles soient) évoluent à leur tour et constituent un système de variables. La classe de la série dépendra des variables dont elle sera fonction. Quand cette variable est la succession dans l'unité locale (quelle qu'elle soit), on appelle *stade* (lat. *stadium*, en espagnol *etapa*) chacune des synécies en série. Ces stades diffèrent (l'expérience le montre) en stabilité, en vitalité. Ils se montrent souvent en concurrence. J'appelle climax le stade régional de maximum biologique stable. Ce maximum biologique correspond, en dedans de la stabilité (appreciée naturellement par rapport à l'histoire humaine) au maximum de masse, et dans l'égalité de masse, le cas échéant, au triomphe dans la concurrence (donc, la plus grande stabilité). Dans l'Espagne xérotophytique, prise en ensemble, ces stades régionaux de maxima ont, dans l'étage inférieur, la forme de bois sclérophylle, ou, exprimés floristiquement, sont les associations de *Quercus ilex*, de *Q. faginea*, de *Q. suber*, de *Pinus halepensis*, de *P. pinea*, etc. Ces associations-ci, en tous les cas de cette formation-la (comme on préfèrera s'exprimer) sont des climax. Dans les vallées fluviales qui entre coupent ce paysage, le maximum biologique correspond à des bois très différents et comme flore et comme biotype: c'est l'ulmetum, le populetum, le salicetum, etc. Voilà aussi des climax. Arrivés ainsi sans rien préjuger à cette notion, c'est, après, à la recherche inductive de dévoiler et les rapports sériaux de chaque climax et ses liens objectifs avec les facteurs du milieu. Mais la notion de climax, créée par les successionistes, a resisté l'épreuve du sociologisme le plus sevère.

Pas besoin non plus du qualificatif de "final," qui risque d'être pas juste ni relativement. Les associations de déterminées chénopodiacées, plumbaginacées, etc., dont il a été question plus en arrière et qui constituent ces enclaves locales auxquelles doit se réserver la dénomination vulgaire de "steppe salée," aboutissent souvent dans la zonation au tamaricetum. Mais, en continuant dans la même direction, la tamaricetum arriverait aux prises avec l'aire d'un quercetum ilicis et d'un pinetum halepensis par exemple. D'autre part le tamaricetum se met aussi dans les "negas" en concurrence avec la végétation de populetum, ulmetum, etc., qui peuvent, dans des circonstances, le venere et le succeder. Où mettre alors le final et avec quel droit? Avec la conception exposée, pas de

final et pas de problème. Le tamaricetum est aussi une climax, qui peut lutter avec d'autres, et voilà tout.

Ayant conservé au mot formation sa valeur historique, j'ai dû chercher une autre expression pour exprimer l'idée de formation climax créée par Clements. Fidèle aussi aux considerations qui précèdent, je suis d'autre part obligé de parvenir à cette idée par voie purement sociologiste.

En réalité le mot "climax" est employé d'ordinaire, et même par Clements dans la pratique, en deux sens; l'un plus restreint rapporté à une association (p. e. quand on discute s'il s'agit de climax ou de subclimax); l'autre collectif, p. e. la formation-climax, ou les "Grassland Climax," "Sagebrush Climax," etc. Je ne vois pas que ce double usage nuise à la clarté. Climax reste dans les deux cas un qualificatif de "maximum biologique." Mais, pour la rigueur nomenclaturiste, on peut appeler simplement climax à l'association, comme expression abrégée d'association climax, et c'est ce qu'on fait dans la pratique; et aux climax collectives, conclimax (au moins quand on voudra souligner la différence). Je définis la "conclimax": une somme de climax, dont les dominantes a'associent entre elles dans une région commune. C'est, aussi dans ce cas, à la recherche inductive d'en déterminer les connexions écologiques ou géographiques. Ainsi, dans une grand partie de la moitié orientale de l'Espagne la somme de climax dont les dominantes régionales sont *Quercus ilex*, *Q. faginea*, *Pinus halepensis*, auxquelles viennent aussi s'agouter *Pinus nigra* en tant qu'il descend à l'étage inferieur et colin, et avec déterminées préfèrences stationelles *Pinus pinea*, *P. pinaster*, et *Q. suber*, constituent une conclimax.

A l'instar de Clements, et conservant cette valeur historique que d'autres ont respecté aussi, j'adopte pour ces climax collectives la terminaison—ion à la suite du nom des dominantes, ou, pour abréger, des plus typiques exprimées de la façon la plus racourcie. Pour le cas exposé, par exemple, je nomme la conclimax Xero-querci-pinion; non parce que je veux introduir dans le titre une notion écologique, mais parce que xero-quercion est une façon d'embrasser dans un seul mot les deux *Quercus* plus regionalement répandues de la conclimax—*Q. ilex* et *Q. faginea*—qui sont les plus xérophytiques des *Quercus arborescentes* de l'Espagne.

Cette notion de la conclimax se rapproche plus ou moins de celle d'alliance (=Verband) de la nomenclature Braun-Pavillard, où elle est aussi exprimée en -ion. Mais, comme ces auteurs ne concrètent pas d'une façon si tranchée la nature des affinités floristuo-sociologiques qu'ils établissent comme fondement, et ne se rapportent non plus explicitement à la notion de climax, je ne me crois pas en droit d'une identification.

C'est clair par contre que ma conclimax n'est pas le complexeclimax (al. Klimax Komplex, lat. complexum climax) de Braun-Pavillard, car celle-ci se rapporte, à ce qu'il parait, à une seule climax (stricto sensu) commune, et embrasse les notions de climat et de "final."

Je souscris, en respect des valeurs historiques, au mot phase (lat. phasis) inclus aussi dans la nomenclature de ces auteurs; mais je tiens à en donner une définition précise: j'appelle "phase": le stade, ou groupe de stades, rapporté

aux circonstances spéciales qui le déterminent. Ces circonstances peuvent être d'ordre divers. Par exemple: phase de graminetum (point de vue physionomique-floristique), phase ruderale ou arvienne (point de vue écologique), phase initiale (point de vue successioniste), etc.

Enfin j'ai éprouvé le besoin de créer encore deux noms nouveaux: péniclimax et paraclimax.

J'appelle "péniclimax" la climax dont les dominantes sont autochtones, mais où la proportion et disposition de celles-ci, ou même la composition subordonée, ont subi des altérations anthropogènes. Exemples: les climax de 'véga" où le populetum a été favorisé et aménagé; les "montes adhesados," où le sous-bois ligneux a été détruit pour faire place au paturage herbacé, avec presque toujours des aménagements aussi; les bois soumis aux aménagements forestiers, etc.

J'appelle "paraclimax" la synécie qui a les caractères de climax, mais dont les dominantes sont d'origine exotique. Exemples en Espagne: les associations subspontannées de *Phoenix dactylifera* à Elche et à Albatera (prov. d'Alicante); les associations cotières d'Agave et Opuntia, etc.

Le Relevé des Synécies

Comme expression générale de collectivité sociologique (non écologique), soit envisagée du point de vue physiognomique, soit du floristique, j'adopte la terminaison en -etum. C'est la plus ancienne, la plus suivie, la plus simple et la plus claire. La distinction entre formation (p.e. herbetum) et association (p.e. nardetum) est déjà contenue dans le radical. Pas besoin donc de deux désinences, car non sunt multiplicanda entie sine necessitate.

Fidèle au sens rigoureusement physiognomique (et non écologique) de la formation, j'appelle symmorphie (lat. symmorphia) la somme des éléments qui, dans une synécie correspondent au même biotype, et je les nomme au moyen de la même dénomination de celui-ci et la terminaison -etum. Je n'utilise pas le mot synusie, employé déjà dans ce sens par Braun et Pavillard, parce qu'en 1920 ils le prennent de Gams "emendatum," parce que dans la deuxième édition de leur "Vocabulaire" (1925) eux mêmes ne l'utilisent plus, et parce qu'en plus symmorphie a dans son étymologie l'expression de son objet, ce qui n'arrive pas pour synusie.

Pour les raisons déjà exposées, la classification des biotypes n'appartient pas à la géobotanique; mais il rentre dans celle-ci la justification d'avoir préféré une certaine classification comme plus appropriée à un certain pays. Dans les climats froids la protection des bourgeons de remplacement peut devenir à certains égards le point de vue dominant. Dans les pays chauds et secs c'est la protection de la plante entière à l'égard de ces deux aspects hostiles du milieu, qui l'emporte. Sans s'engager en des considérations écologique a risque d'être blâmé d'hypothétiste, la végétation méditerranéenne est éminemment ligneuse: le problème du revêtement ou non par un rhytidome de tissus morts se pose donc en premier lieu. Dans la même région la plupart des plantes annuelles doivent accomplir leur cycle végétatif avant la saison plus chaude, qui leur serait

hostile: il ne leur saurait donc convenir le qualificatif des thérophytes. C'est pour des raisons de cet ordre, que je n'ai pas utilisé la classification biotypique de Raunkiaer (très savante et très utile pour d'autres vues), et je lui ai préferé une autre dont les fondements ont été plus généralement adoptés, à travers les différences des catégories résultantes, depuis les sept de premier ordre de Grisebach (1872) aux quatre de Clements (1920), et qu'on pourrait, à mon avis, schématiser de la sorte, réduite a des termes symmorphiaux:

<table>
<tr><td></td><td>GRAMINOIDETUM
Protection par silisation, etc.</td><td></td></tr>
<tr><td>Lignetum
Prorection axile par un étui de tissus morts</td><td></td><td>Herbetum</td></tr>
<tr><td></td><td>CRASSICAULETUM
Protection par emmaginement d'eau</td><td></td></tr>
</table>

Comme toujours, c'est l'homme qui divise; mais *natura non facit saltus*: quelques graminoidées et quelques succulentes rhytidomisent aussi plus ou moins son ace, surtout à la base; de ces deux groupes on passe sans coupures brusques à l'herbetum; et le même arrive pour le lignetum, puisque, non seulement il y a la forme de sous-arbrisseau, mais des espèces qui se montrent tantôt herbacées tantôt sous-frutescentes.

Mais cette première division, que tant d'autres ont, plus ou moins, suivie, n'a eu, réellement, en vue que la végétation hautement évoluée, telle que l'a permise un milieu suffisamment favorable, lui offrant une certaine somme de chaleur et un sol (stricto sensu), produit à la fois des facteurs géologique, climatique et biotique. Or, dans la double évolution des plantes et de la physiographie terrestre, la végétation commence avant cet optimum général du milieu, dans des conditions que j'appelelrai primordiales. Dans ces conditions primordiales la végétation offre des formes qui, naturellement, ne se laissent pas bien enchâsser dans les termes de cette division là. Il faut donc ne pas s'y entêter. Avec ce fondement tout-à-fait naturel, j'appelle "proteretum" la somme de formes de végétation propres des "conditions primordiales" du milieu. En opposition j'appelle "hysteretum" tout l'ensemble de formes développées dans les conditions d'optimum général. Le proteretum se montre exclusif ou dominant: (1) Du point de vue écologique dans les deux formes de milieu substratif antérieures à la terre ou sol (stricto sensu): d'une part l'eau (ou la glace); d'autre la roche non encore transformée en terre, comme le rocher. (2) Du point de vue géographique dans le climat offrant un minimum encore trop bas de température: les calottes polaires et les premières terres subpolaires à formations de tundras.

Du côté du rocher le proteretum, dans le sens le plus rigoureux du mot, se composerait exclusivement de thallophytes (parmi lesquelles les lichens jouent un rol bien important). Mais, étant donné que les mousses les suivent de si près, et qu'elles se contentent d'une portion de terre si minime et si élémentaire, qu'il a été question de s'ils peuvent même s'en passer, et surtout pour le point de vue physiognomique, qu'ils continuent à prendre de l'eau, comme les thallophytes, par toute la surface, c'est après les muscinées que je trace la délimitation du proteretum.

Du côté de l'eau la délimitation est moins tranchée, ce milieu ayant admis des formes plus évoluées. Mais, comme le détail ce problème appartient au domain écologique, je m'y rapporte plus loin.

Je ne prétends pas, il faut bien y faire attention, que toute série sur un sol nouveau doive commencer toujours par un protéretum, dans les sols sédentaires, comme les calcaires et les marnes magnésifères du miocène et du crétacé du plateau espagnol, les psammites rouges si fréquentes dans le trias tout proche, le granit, le gneiss et les schistes de la Cordillère Centrale, etc., la lithosérie peut bien se reconnaitre à partir du proteretum. Mais dans les sols colluviaux, comme les sables quaternaires au pied de cette même Cordillère, quand des causes accidentelles originent une étendue de sol tout-à-fait nu, ce sont, au moins quant à la végétation macroscopique, des syphonogames les "pioneers" les plus habituels. Et je ne prétends non plus que, dans toutes les synécies, le proteretum représente toujours la première végétation établie sur l'unité locale dans l'ordre du temps; car il arrive souvent le contraire, par exemple pour les mousses et les hépatiques qui ont besoin de l'ombre des plantes supérieures ou du sol humifère que celles-ci contribuent à former et entretenir (et cela même en reconnaissant que ces nouvelles conditions créent un sol en quelque sorte nouveau par rapport à l'antérieur). Je ne fais que donner un nom à l'ensemble de formes qui sont uniques à se développer dans les habitats primordiaux.

Il y a encore des types biologiques non rapportables ni au milieu primordial ni a celui optimal. Car ces deux groupes de milieux coincident à être des milieux physiographiques. Il y en a qui habitent des milieux biologiques. J'appelle symphytetum la somme de formes propres au milieu biologique; et je le divise, du point de vue biologique même, en epiphytetum, paraphytetum et saprophytetum. Quant aux lianes, le fait de s'appuyer ou s'accrocher à d'autres plantes les rapproche de l'epiphytetum, mais ne leur empêche pas d'enraciner par elles mêmes dans le sol et d'y puiser leur nourriture: je les rapporte donc au groupe respectif dans l'hysteretum (herbetum, lignetum, etc.).

On peut donc tracer de cette sort le tableau des catégories symmorphiales superieures:

- (A) Oecophytetum
  - I—Proteretum
    - Hydroproteretum
    - Pezoproteretum
  - II—Hysteretum
    - Herbetum
      - Annuiherbetum
      - Bienniherbetum
      - Perenniherbetum
    - Graminoidetum
      - Annuigraminoidetum[4]
      - Biennigraminoidetum
      - Perennigraminoidetum
    - Crassicauletum
      - Herbicrassicauletum
      - Lignicrassicauletum
    - Lignetum
      - Suffruticetum
      - Fruticetum
      - Arboretum
- (B) Symphytetum
  - Epiphytetum
  - Paraphytetum
  - Saprophytetum

[4] Quand l'ensemble de formes graminoidéees ne comprend que des graminées, je racourcie le nom en graminetum.

Quoique j'ai dû parler du milieu, je ne fais aucune confusion entre écologie et physiognomie. J'ai commencé en établissant la cohabitation comme point de départ, et je rapporte la classification de formes biologique aux trois sortes de lieux habités qui montre la nature.

Dans le tableau qui précède je ne fais pas question de ce que les unités de chaque colonne correspondent exactement au même degré divisionaire. Le tableau n'a d'autre but qu'exposer dans une forme brève et claire les unités des premières catégories. Entrer dans les subdivisions ce serait une longue tâche qui demande un travail spécial. Ces subdivisions ne peuvent former un tableau unique et rigide. Elles différent d'après le point de vue qu'on a besoin de prendre en chaque cas. S'il est, par exemple, biophénologique, il serait question d'un lignetum estivifolié, pluvifolié, perennifolié, etc. S'il est morphologique, on pourrait distinguer p.e. dans le graminetum un fascigraminetum, un caespitigraminetum; dans le lignetum les subdivisions de planifolié, aciculifolié, etc. S'il est stratigraphique, il y aurait lieu aux dénominations de giganti-arboretum, elatiarboretum, parviarboretum, fruticuletum, lignuletum, herbuletum, et d'autres pareilles. Et ainsi de suite.

Envisagée la synécie comme association, ils se posent surtout, à l'égard de sa composition floristique, les problèmes de la quantité et de la sociabilité.

Pour ce dernier je me suis rattaché à l'idéologie de Clements. Mais, puisque, par respect aux valeurs historiques, je ne puis accepter pour le mot association aucun sens spécial, j'ai dû introduir des modifications. Mis dans cette voie, j'ai tenté d'universaliser la nomenclature de Clements, en l'exprimant en latin et en faisant rentrer dans la régularité de la désinence toutes les notions. J'ai laissé pour les stades sériaux la terminaison -es; et pour les climax, ne pouvant employer -atio, quisque le mot associatio doit conserver son acception historique générale, j'ai adopté la désinence en -etas. La nomenclature, avec les abréviations et la signification respective, reste donc comme il suit:

Climax	Ante-climax	Signification
Consocietas (CS)	Consocies (cs)	Dominance d'une seule espèce
Associetas (AS)	Associes (as)	Dominance de plusieurs espèces
Societas (S)	Socies (s)	Distribution par toute la synécie
Grex (GR)	Gregies (gr)	Accumulation dans une tâche ou peu de tâches
Cumulus (CM)	Cumulies (cm)	Accumulation en groupes très petits
Sporadium (SP)	Sporadies (sp)	Représentation isolée par un individu ou pas beaucoup plus

Dans mon Avancement sur la Steppe Centrale de l'Espagne, j'avais adopté les abréviations Cs, As, etc. pour la climax. Mais après j'ai réflechi qu'il voudrait mieux les écrire en majuscules, CS, AS, etc., de cette sorte les abréviations en majuscule et minuscule—As, Cs, Sc, Gr, etc.—pourraient être reservées pour le cas ou l'on ne veule exprimer (pour n'être pas la chose encore mise en clair, par exemple) s'il s'agit de climax ou non. Dans ce cas il n'y aurait pas inconvénient à se servir des dénominations en -atio (consociatio, associatio, sociatio, gregatio, cumulatio, sporatio). Le mot associatio aurait alors un sens général et un autre plus restreint; mais ce défaut n'a pas de portée dans la pratique,

puisque toute "associatio" stricto sensu est aussi une "associatio" lato sensu, et ce double sens est une conséquence de la valeur générale de la désinence en -atio, en harmonie avec le nom de l'unité générale Associatio (Association).

J'ai combiné ces dénominations de sociabilité pour exprimer des nuances. Par exemple, *Societas gregaria* (S^{gr}) voudrait dire que l'espèce se répète en grèges par toute la synécie; *grex cumularis* (GR^{cm}) qu'il s'agit d'une *grex* de *cumuli*; *socies sporadica* (S^{sp}) que les individus se répètent très isolément; etc.

En honneur des valeurs historiques je crois qu'il serait préférable d'éviter la dénomination de famille (*familia*), ou toute autre tirée de la même étymologie, pour les colonies à une seule espèce. Parallèlement à ce qu'on fait avec la société dominante, on pourrait subdiviser la colonia en concolonia (cc), pour celle à une seule espèce et accolonia (ac) pour celle à plusieurs. Ici, par la nature de l'idée, pas besoin de majuscules.

Ce système permet de réunir dans un seul symbol la sociabilité et la quantité. J'ai employé, par exemple, cette notation:

s″	*socies* très dense
s′	" dense
s	" assez éparse
S	" très éparse
S	" sporadique

Si l'on veut exprimer l'idée numériquement on peut se servir de chiffres: CS^4, AS^5, S^2, etc. Ou bien, en conservant les nuances de sociabilité: S_3^{cm}, s_1^{sp}, etc.

Ce système est compatible avec les différentes notions quantitatives. Celle de constance dans le sens de l'école d'Upsal et la méthode des carrés avec laquelle, on la mesure, sont très appropriées pour déterminer et exprimer graphiquement ces notions de sociabilité.

En honneur de la clarté, il serait à désirer pourtant l'abolition du mot constance dans ce sens, ainsi que celle du mot dominance dans celui de Deckungsgrad. Je proposerais: constance (lat. constantia) pour le sens synthétique (Konstanz de Brockmann et de l'école de Zürich); densité (densitas) pour le sens analytique quantitatif suédois et la correspondante détermination par la méthode des carrés; ainsi que fréquence (frequentia) comme il est déjà d'usage général, pour le résultat de la méthode de Raunkjaer. Le mot dominance (dominantia) par des raisons de prélation, doit être restitué au sens de sociabilité dans lequel on n'a jamais cessé d'employer des expressions comme celle d'espèces dominantes et d'autres pareilles. On peut parler au même titre de la "dominance" d'une symmorphie sur les autres; et conserver le rapport étymologique avec le moit domaine. Pour le sens de Deckungsgrad j'emploie le mot expansion (expansio).

Mais, à ce propos, j'ai cru utile de distinguer une expansion horizontale ou projection sur le sol (Deckungsgrad), et une expansion aérienne. Je mesure celle-ci, dans les formes non arborescentes, par le volume embrassé par la surface circonscrite à la plante, en le considérant comme un élipsoïde. Ja calcule ce volume par les trois axes, ce qui exige prendre des mesures sur un nombre suffisant d'individus, et apprécier l'abondance de ceux-ci. Je rapporte après les résultats

à une unité de surface donné, l'hectare, quoique le représentant d'association soit souvent beaucoup moins étendu.

Pour faire voir la partée de cette notion, je vais me rapporter à une synécie décrite dans mon Avancement sur la prétendue Steppe Centrale de l'Espagne: celle de la photogravure 13–III. La grande masse de la végétation y correspond à deux espèces: le *Thymus zygis*, qui forme une consocies régionale très répandue; et la *Salsola vermiculata*, qui concurre avec lui dans l'endroit comme un phénomène local restreint. Selon que l'on envisage l'expansion horizontale ou celle aérienne, le résultat est fort divers.

	Expansion horizontale per cent	Expansion aérienne m^3 par Ha
Thymus zygis	25.3	197
Salsola vermiculata	4.8	208

À ne prendre en considération que le Deckungsgrad, on continuerait à qualifier l'association de "consocies de *Thymus zygis*" avec "gregies sporadica de Salsola." Mais l'expansion aérienne montre que la concurrence est arrivée à équilibrer dans cet endroit le domaine des deux espèces et qu'il s'agit d'une "associes" locale ou stationelle.

On conçoit bien de raisons en faveur de l'expansion aérienne. L'air étant aussi une partie du milieu, le volume qu'on en occupe, en tout et dans chaque strate, doit bien avoir une valeur, puisque la masse a des rapports avec la concurrence et avec la réaction sur le milieu. D'autre part cette masse vivante a été formée aussi aux dépens des éléments de nutrition fournis par le sol.

La concurrence et, comme résultat, la dominance (dans mon sens) étant exprimées dans la masse totale de végétation nourrie par le milieu, il y a une raison en plus pour ne pas restreindre la dénomination de dominance à la surface occupée.

Très fréquemment, même dans des synécies très riches en espèces, comme il arrive déjà souvent dans les pays méditerranéens, la plupart de la masse végétale se partage, comme dans l'exemple ci-dessus, dans un petit nombre d'espèces, et c'est dans celles-ci qu'il convient de porter à l'extrème la précision dans les releves quantitatifs. De ce point de vue on peut résumer comme il suit la perspective de la synécie, en rapportant les qualificatifs à "végétation" ou à "espèces," d'après le point de vue à prendre.

Dominans (Dominante) . . . . } Major (Majeur ou Principal)
Subdita (Subordonnée) { Concomitans (Concomitante) . . . . . . } Major (Majeur ou Principal)
Subdita (Subordonnée) { Addita (Additionelle) . . . . } Minor (Mineur)

La végétation additionelle est formée par la somme d'espèces qui, par sa petite masse spécifique ou par son degré minime d'abondance, ne représentent qu'une proportion insignifiante dans la masse végétale totale (toute considérable que puisse être, du point de vue floristique, l'importance de chaque espèce).

Enfin, pour l'étude de la concurrence j'ai éprouvé le besoin de prendre en considération, outre les dominantes de chaque synécie, la dominante ou dominantes de chaque symmorphie; et, outre les rapports quantitatifs des espèces, ceux des symmorphies entre elles, et aussi ceux des différents groupes d'espèces ayant des significations écologiques ou géographiques diverses. Comme exemple je me rapporte à la synécie gypsosériale représentée dans la photogravure 19 de mon "Avancement . . . ." citée et décrite la même dans les pages suivantes. On reconnait là la concurrence, non seulement des espèces entre elles, mais de deux associations: l'une stationelle, dont la dominante est la *Gypsophila struthium*, l'autre régionale (dénomination préférable à celle de climatique), dont la dominante est le *Thymus zygis* et qui dans ce cas a le dessous.

Je laisse pour une autre occasion de m'occuper des caractères synthétiques des synécies.

Systématique des Synécies

Étand donné qu'il faut un mot applicable en commun à toutes les unités inférieures à celle d'association, je propose de laisser ce sens général ou mot variante, qui, dans la practique, y est employé souvent.

Pour les différentes unités il serait désirable de fixer une nomenclature en gradation comme on l'a fait pour la systématique des espèces; par exemple: association, sous-association, type, subtype, etc.; puisque le mot "type" vient aussi utilisé dans la littérature rapporté à des différences floristiques. En plus, puisque ce mot a en composition (Waldtypus) un sens déjà fixé par Cajander dans sa classification des bois par la sous-bois, il faudrait, en l'employant seul, lui donner un sens compatible avec celui qu'il a dans ce cas particulier. En tout cas je ne penche pas pour appliquer à la systématique des associations les mêmes noms (comme on est arrivé à le proposer) qu' à celle des espèces, puisqu' en somme il s'agit de choses différentes.

Il m'a résulté aisé d'employer le mot facies (comme unité subordonnée d'association) dans ce sens: la variante ou groupe de variantes dont les limites sociologiques coincident avec des limites topologiques. Avec cette définition on ne préjuge rien sur la nature des rapports écologiques ou géographiques. Ce sens je le crois très légitime, et même exigé par la conservation de valeurs historiques. La définition que Lorenz donne à ce mot, en l'employant en 1863, contient déjà ces deux notions de différence floristique et de rapport avec le milieu.[5] La dernière n'a pas été toujours maintenue; mais celle du sens floristique a été conservée par presque tous les auteurs qui se sont depuis lors servis de ce mot: Drude, Schimper, Pound et Clements, Schröter, Brockmann, etc. Je trouve donc trop en contradiction avec les valeurs historiques, le sens qui lui est attribué dans la nomenclature Braun-Pavillard, d'après laquelle "les facies se distinguent seulement par des différences quantitatives plus ou moin notables." Pour la notion ainsi exprimée je les inviterais à préférer le mot nuance ou quelque autre de valeur pareille.

[5] ". . . . Vegetationsformationen (en réalité des associations) oder Unterabteilungen derselben, betrachten vom Standpunkte der Lokation und der bedingenen physikalischen Agentien."

C'est donc en employent le mot facies dans le sens que je viens de justifier, que j'ai parlé, par exemple, pour l'association de *Quercus ilex* au centre de l'Espagne, d'une facies calcicole (avec sous-bois de *Q. coccifera*, *Genista scorpius*, *Rhamnus lycioides*, *Laureola gnidium*, *Rosmarinus officinalis*, etc.); d'une facies gypsicole (avec addition, aux éléments floristiques régionaux, d'*Ononis tridentata*, *Gypsophila struthium*, *Lepidium subulatum*, *Helianthemum squamatum*, *Herniaria fruticosa*, etc.); d'une facies silicole, qui, prise en ensemble embrasse des types différents (celui à *Retama sphaerocarpa*, celui à *Cistus ladaniferus*, etc); d'une facies basale ou sous-montane; etc.

Quant aux multiples d'association j'ai proposé déja et défini le mot de con-climax, en substitution de la "formation-climax," ainsi qu' exposé sa différence à l'égard du "complexe climax" de Braun-Pavillard.

Le mot complexe, introduit en 1917 par Du Rietz et presqu'au même temps par Nichols, mérite bien son succès. Il ne cache en lui même aucun parti pris, et, étant largement applicable, permet de remplir aisément plusieurs vides. On pourrait définir la notion de complexe, prise dans un sens général comme une somme d'associations en rapport. Il dépendrait du point de vue pris dans chaque cas, de préciser la nature du rapport en question. En comparant cette définition avec celle que j'ai donné plus haut de série, le complexe apparait comme une notion générale de laquelle la série est un cas particulier. La série est un complexe à rapport antéro-postérieur. Les deux notions ne seraient donc pas opposées; mais harmoniques. Pour DuRietz le rapport de son "Assoziationskomplex" est exclusivement, ou avant tout, sociologique, de même que celui des espèces dans une association. Je trouve que justement, dans un cas comme dans l'autre, c'est ce rapport direct, de plante à plante et association à association, ce qu'il y a de plus difficile à demontrer; car ce que nous voyons directement dans le fait simultané c'est la cohabitation et pas autre chose. Dans tous les complexes que je connais, je trouve toujours un rapport topologique ou même aussi chronologique, qui, au fond a servi de guide, plus ou moins apparente, pour les distinguer. Ainsi, pour les deux exemples cités par Du Rietz comme des plus typiques, la tourbière se rapporte à la succession, et la végétation des rochers côtiers a besoin d'être définie par l'écologie. Dans la définition de Nichols on reconnait franchement le fondement topologique, en rapportant l'association-complex à l'habitat-complex.

Quel que soit le lien qui puisse y avoir entre les associations, il est évident qu'il y a bien des complexes rapportables à la topologie; et, par conséquence, que l'on peut envisager des comp'exes écologiques et des complexes géographiques, et, dans ce dernier groupe, des complexes locaux et régionaux, ceux-ci subdivisibles d'après les unites geographiques.

Ainsi cette notion créée par un sociologiste des plus rigides, ouvre justement le pas à l'établissement des rapports de la synécie (étudiée d'abord "en soi-même dans l'ordre méthodique) avec le milieu.

Le Milieu

Ainsi que j'ai adopté la désinence-etum comme caractéristique des synécies dans leurs sens sociologique, j'adopte, dans ma terminologie latine, la composi-

tion finie en-phytia pour exprimer le groupement végétal rapporté au milieu. Fidèle au respect des valeurs historiques, je garde ici le parallélisme avec les classes écologiques de Warming terminées en-phytes.

C'est après avoir constaté la constance de rapports d'habitat entre une synécie préalablement étudiée, et une stztion déterminée, que je me crois en droit de qualifier la synécié de "xerophytia," "halophytia," "gypsophytia" ou quoi que ce soit.

Pour les mots de rapport, applicables ainsi aux espèces qu'aux synécies, j'emploie la terminaison en-cole (lat.-cola), qui, en soi-même, exprime seulement le fait évident de l'habitation: salicole, xéricole, rupicole, etc. . . . . La taxation de la valeur écologique vient après, en faisant distinction, pour chaque qualification en-cole, de trois degrés:

———phile, exclusive (au moins dans son plein développement synécial) de cette station.
sub———phile, non exclusive, mais avec plus ou moins de préférence pour elle.
———ade, adaptable au même milieu, mais sans de préférence claire.

Ainsi, dans les véritables halophyties de l'Espagne centrale, qui s'y montrent comme enclaves, déterminées par la topographie en rapport avec le climat, on peut citer comme exemples d'espèces halophiles typiques: *Lerchea fruticosa*, *Inula erithmoides*, *Puccinellia distans*, *P. leptophylla*, *Agropyrum elongatum*, *Juncus maritimus*, *Gypsophila tomentosa*, *Limonium duriusculum catalaunicum*, *L. ovalifolium*, *Plantago maritima*, *Sonchus crassifolius*, *Salicornia herbacea*, *Sphaenopus Gouani*, *Salsola soda*, *Chenopodina maritima*, *Microcnemum fastigiatum*, *Frankenia pulverulenta*, etc. Apparaissent seulement comme subhalophiles bien caracterisées: *Atriplex halimus*, *A. glauca*, *Aleuropus littoralis*, *Juncus acutus*, *Samolus Valerandi*, *Cressa cretica*, *Sonchus aquatilis*, *Alsine canadensis*, *Polypogon maritimus*, *Hordeum maritimum*, *Salsola kali*, *S. tenuifolia*, et, d'autres. Enfin, parmi les exemples très nombreux d'haloades, je citerai: *Ephedra distachya*, *Lavatera triloba*, *Frankenia Reuteri*, *Holoschoenus pectinatus*, *Zannichellia palustris*, *Ranunculus aquatilia*, *Peganum harmala*, *Limonium dichotomum*, *Plantago albicans*, *Centaurea calcitrapa*, *Crypsis aculeata*, *Polypogon monspeliensis*, *Atriplex hastata*, *Chenopodium rubrum*, *Limonium echioides*, *Xanthium strumarium*, *Asteriscus aquaticus pugmaeus*, *etc.*

D'une façon analogue on peut distinguer en Espagne des espèces gypsophiles, subgypsophiles et gypsoades. Or: la flore gypsophile (dont j'ai cité des exemples plus haut) est tout-à-fait différente de la flore halophile. La flore gypsophile se montre en plein développement écétique sur des sols dont la tenue en sels soubles est très basse et du même ordre que celle des sols calcaires et marneux à climax de xero-quercetum, et même que celle des "vegas" à végétation mésophytique de populetum. Cette flore gypsophile se mêle à la végétation régionale du xero-quercetum climax en en caractérisant une facies.

Willkomm dressa de longues listes d'espèces espagnoles qualifées par lui d'halophiles sans appuyer cette qualification sur aucune analyse du sol. Or, les espèces qui habitent d'une façon préférente ou exclusive les sols à haute tenue

de sels solubles, sont beaucoup moins nombreuses. Dans les listes de Willkomm il y a de vraies halophiles (une minorité), des subhalophiles, des haloades, et encore d'autres espèces pas même haloades, mais tout simplement xérophiles, gypsophiles rudérales, etc. La grande étendue régionale attribuée par lui aux steppes salées en Espagne comporte la confusion dans un même tout de la végétation vraiement salicole avec la gypsicole, une grande partie de la calcicole, de la rudérale, etc. C'est un exemple de l'erreur où peut conduire le préjugé écologiste, et justifie la critique du sociologisme contre ce partie pris.

Je me suis remis plus haut a l'étude de l'hydrophytia, pour décider sur la limite du protéretum hydrosérial. J'établis dans l'hydrophytia cette sériation de formes en rapport écologique (abstraction faite de toute considération systematique florale):

(1) Plankton divisé en epiplankton (surnageant), hypoplankton (sours-nageant) et thetoplankton (posé au fond).

(2) Pleon ("pleuston" de Schröter); divisé en epipleon (surnageant, "pleuston" de Kirchner), phypopleon (submergé), et heteropleon (alternativement surnageant et submergé).

(3) Hydrostadion: pleinement aquatique, divisé en ploadostadion (à partie supérieure surnageante), et baphostadion (tout-à-fait submergé). Pour les fragments des plantes de l'hydrostadion, qui puissent faire croire à un pleon j'emploie la dénomination de pseudopleon.

Stadion
Station fixée dans un fond submerge

(4) Helostadion: submergé seulement à la base (et pas toujours pérennement), dans le reste élevé dans l'air.

Les trois premières catégories forment la véritable hydrophytia; la quatriéme l'helophytia. A celle-ci fait déjà suite la pezophytia ou ensemble de végétation émergée ("terrestre" par opposition à "aquatique").

Le formes le l'hydrostadion, quoique fixées dans le fond, se rapprochent physiognomiquement de celles du pleon, étant adaptées comme celles-ci à la vie dans l'eau. La division la plus tranchée tombe donc entre l'hydrostadion et l'helostadion. C'est donc là que doit finir le protéretum.

Cette fois il ne m'a pas été possible de respecter tout-à-fait les valeurs historiques, dû au besoin de composer un ensemble harmonique auquel ces valeurs ne se prêtaient pas assez. J'ai du diviser le benthos entre les catégories 1 et 3, et grouper le michobenthos avec le microplankton avec lequel il coincide en station passive et en types physiognomiques. Ma notion de pleon ne correspondant pas à celle de pleuston dans son sens originaire (Kirchner), et ce dernier mot ayant été employé en deux sens différents, j'ai cru qu'il y avait droit à le substituer par "une autre forme du même mot," plus satisfaisante encore étymologiquement et plus harmonique avec le reste du système, et delivre de tout double sens.

La même méthode inductive suivie pour la qualification écologique des espèces et des synécies, doit être appliquee à la distinction des étages dans la clisérie.

Les limites et les dénominations des étages doivent être dérivées de la connaissance préalable de la végétation même. En prenant comme exemple, en vue de sa latitude moyenne, la Cordillére Centrale, et, pour plus concréter, dans la moitié orientale de sa masse granitique-gneissique, on y reconnait, comme dominantes des climax et sub-climax principales qui se succèdent, les espéces suivantes:

Quercus ilex: cette espèce qui, en consociétas ou en associétas, caractérise aussi les étages inférieurs, monte aujourd'hui, dans plusieurs points, jusqu'à des hauteurs entre 1200 et 1300 m.; et, dans des endroits, encore plus haut.

Pinus pinaster: cette espéce, qui se montre aussi comme cotière, apparait ici, en consociétas, vers les 700 m., et monte un peu plus haut que les limites ordinaires actuelles du quercetum ilicis. En bas il s'associe en outre, comme également la *Q. ilex*, avec la *Pinus pinea*; en haut avec la *Q. toza*.

Quercus toza: les consociétates de cette espéce, déjà caduci-foliée, commencent à se montrer, dans plusieurs endroits, assez bas, avant les 1000 m., et même avant les 900; très frèquemment elle cède déjà absolument la place, dans l'état actuel des choses, à l'espèce suivante vers les 1500 ou les 1600 m; mais dans l'autre lieu on peu constater, aujourd'hui même, que sa véritable limite climatique peut dépasser les 1700.

Pinus silvestris: on reconnait des nos jours la plus haute présence, ainsi de sa consociétas que de ses derniers individus isolés, entre 2100 et 2200 m. a Siete-Picos et a Peñalara, dans des conditions topographiques non optimales (dernier individu isolé, à l'est du Collado de Dos Hermanas, vers 2170 m).

Le *Spartocytisus purgans* et d'autres génistées, et surtout la Juniperus communis montana (-nana W.), après avoir formé des sociétates dans les sous-bois et des préclimax dans les clairières, dominent la végétation ligneuse des hauteurs au delà des arbres.

La *Nardus stricta* joue à l'égard du pinetum silvestris, et puis s'épanouissant en pelouses dans les hauteurs découvertes, et partageant l'espace avec les derniers buissons, ainsi qu'avec d'autres graminées (*Festuca* sp. var., *Deschampsia flexuosa*, *Agrostis delicatula*, etc.) le même rôle que dans les montagnes de l'Europe moyenne.

Enfin le lichenetum, seul ou entremêlé de muscinetum, et entrecoupé de vegetation fissuricole, préside au complexe des crêtes rocheuses.

Les bois originaires n'ont été guère conservès dans les montagnes plus que dans la plaine: si la denomination de "steppe ligneuse" était admisible, on pourrait la faire bien monter en hauteur. Sur le sol granitique et gneissique des parties basses de cette cordillere, il y a bien des étendues de tomillar, dont la dominante est le même *Thymus zygis* de ceux de la prétendue "steppe centrale" calcicole. Pourtant dans les "sierras" personne n'a mis en doute que le végétation originaire naturelle fusse le bois, au moins jusqu'à une certaine hauteur. L'état déboisé des montagnes montre donc jusqu'à quel point peut arriver la dévastation humaine, et éclaire le problème de la succession dans la plaine, en renforçant l'attribution d'un caractère subsèrial à la végétation ligneuse calcicole et gypsicole qualifiée à tort de steppe.

Le problème de la clisérie est bien plus compliqué de ce qui apparait en ne prenant en considération que le rol des espèces citées. On pourrait y ajouter, par exemple, la classification d'après le système de Cajander, fondé sur le sous-bois. Mais, en se tenant à ce qu'est dit, comme exemple de méthode à suivre (la seule chose dont il s'agisse ici), l'échelonement des dominantes et associations citées nous mène inductivement à une première distinction d'étages.

Un 1^r^ étage, que je qualifie de basal, caractérisé encore par la présence dominnate de la *Quercus ilex*, espèce perennifoliee et scérophylle, avec laquelle peut concurrir la *Pinus pinaster*.

Un 2e étage, où la *Quercus toza* devient dominante. Le caractére planicaducifolié de cette espèce permet pour cet étage la calification de montagnard, à l'instar d'un étage analogue dans d'autres pays.

Un 3e étage où la *Q. toza* disparait à son tour et la climax appartient à la *Pinus silvestris*. Comme cet étage a donc le même caractére physiognomique que celui califié dans l'Europe moyenne de sub-alpin, il n'y a pas d'inconvénient à lui étendre ce nom. Il faut pourtant prendre en considération, qu' à cause du déboisement, une grande partie du domaine exclusif de cette climax est aujourd'hui à nu, et qu'à son tour, de considérables étendues de la consociétas de *P. silvestris* se montrent à des hauteure inférieures, c'est-à-dire dans l'étage de la *Quercus toza*, ayant substitué cette espéce, ou la dominant comme sous-bois, ou en englobant les derniers fragments et termoins. (Le déboisement a été encore plus acharné dans la moitié occidentale, à tel point, qu' au versant N. les Sierra se Grédos, les parties les plus hautes du pinetum silvestris se montrent plus bas que les témoins les plus hauts de l'ancienne consociétas de *Quercus toza*.)

Enfin un 4e étage, où l'absence d'arbres et les pelouses de *Nardus stricta*, et d'autres graminées, offrent un paysage d'aspect alpin. Mais dans l'ensemble qui porte aujourd'hui cette empreinte, il se pose le problème de mettre en clair où a-t-on à faire a de véritables climax, et où ne s'agit-il pas que les subclimax.[6] Et c'est seulement au domaine des premières qu'il correspondrait en propre la dénomination d'étage alpin.

L'état de déboisement le tardive connaissance de l'hypsometric, et le parti pris géographique, contribuent à expliquer le frappant désaccord des auteurs à dénommer et déterminer topographiquement les étages de la cliserie espagnole. Ainsi, pour le même Cordillére Centrale, Pascual (1858) met à 1080 m. la limite supérieure de l' étage qu'il appelle "montano" et qu'il caractérise par la *Quercus toza* et le chataignier à la fois; et à 1660 m. (encore en bas du tozetum actuel le plus haut) la limite supérieure de l'étage qu'il appelle sub-alpin ou de la *Pinus silvestris*. Cutanda (1861) place sa "zona montana inferior o de las coniferas" (sic) entre 1115 et 1672 m. Secall (en 1889) trace à 1500 m. la limite entre ses régions "montañosa" et alpine. Willkomm (1896) califié de sub-alpin l'étage entre 1150 et 1800 m. (qui, comme nous avons vu, appartient encore, pour la plupart, à la *Quercus toza*). Làzaro (1906) se rapproche plus de la vérité

[6] C'est bien aussi le cas dans la moitié occidentale, pour le mosaïque de "piornal" et festucetum elegantis si caracteristique des hauteurs de Grédos.

en portant jusqu'à 1600 m. sa "zona montana" et a 2000 m. celle qu'il appelle "subpirenaica;" et encore plus Mazarredo (1911) en indiquant que la *Quercus toza* arrive parfois jusqu'à 1770 m; et Laguna s'en était rapproché déjà, en 1870, en assignant les 2100 m. comme limite supérieure de la *Pinus silvestris*, quoique ça reste encore un peu au dessous de la réalité même actuelle.

Comme unités chorologique j'emploie l'échelle de Flahault, qui est aussi celle adoptée dans la nomenclature Braun-Pavillard, et devient d'un usage de plus en plus général. Elle s'imposait en outre par le besoin de mettre en rapport les divisions phytogéographiques de l'Espagne avec celles des pays voisins.

Seulement, étant donné le besoin d'un terme général, j'ai cru que le plus indiqué pour cet usage était celui de région. Il a été de longtemps le plus employé et celui qui a été appliqué aux significations spéciales les plus diverses. Il est implicitement voué à cette usage par le même Flahault, avec Schöter, dans leur rapport au Congrès de Bruxelles (8 k. de la Proposition).

En remplacement de région, je propose pour la division de premier ordre le mot latin *Geophytis* (qui dans quelques langues neolatines doit être connaturalisé dans la forme de l'ablative: en espagnol "Geofitide"). Ce mot a les avantages suivants: il renferme les deux notions de milieu terrestre et de plante, qu'il s'agit de mettre en rapport; et il garde parallélisme de forme avec les grandes divisions analogues proposées en géographie et paléogéographie: Antarctics, Atlantis, etc. On dirait donc, par exemple, la *Geophytis* méditerranéenne.

Il va sans dire que ce doit être le contenu géobotanique, et non le caractère géographique le fondement de la division du globe en géophytides; et qu'il arrive de même pour les autres unités inférieures.

Ainsi celle de deuxième degré, ou domaine, se dérive aisément de la notion phytosociologique que la justifie, et même garde le parallélisme étymologique d'expression avec la notion de dominante, qui y intervient comme fondamentale. Je considère le domaine (dominium) comme l'aire géographique correspondant à une conclimax. En égard à la définition de conclimax donnée plus haut, on peut définir directement le "domaine": L'aire géographique commune des dominantes régionales qui s'associent entre toutes. Le domaine peut se dénommer par la conclimax qui le justifie: par exemple, comme une division de la Géophytis méditerranéene, le domain du xero-querci-pinion. Ce domaine occupe la plus grande partie de l'Espagne orientale. Mais il faut ne pas troubler ces unités naturelles avec des frontières politiques. Le domaine dont il s'agit s'étend bien au delà des alignements douaniers. Ainsi, ce que Flahault appelle, pour abréger le "domaine méditerranéen français" est la partie française du domaine du xero-querci-pinion méditerranéen.

Le domain, étant une unité chorologique, et non sociologique, embrasse non seulement les climax avec ses prisériés et ses subséries (c'est à dire les coséries de Clements, les complexes climax de Braun-Pavillard), mais aussi des enclaves, dont les dominantes n'arrivent à avoir une étendue qui mérite le qualificatif de "regionale." Tel est le complexe domanial. De la même façon qu'on nomme

chaque association par ses dominantes individuelles, on doit accepter la dénomination du domaine ou de son complexe par ses dominantes collectives.

L'échelle des unités chorologique sera donc:

Geophytis, Domaine, Secteur, District, Sous-district.

A chacune de ces unités correspond un complexe, caracterisé par des dominantes sociologiques et floristiques determinées.

Dans quelques cas on peut avoir encore besoin de quelque unité intermédiare Dans une bande côtière assez large, car l'espèce monte assez haut, du domaine du xeroquerci-pinion méditerranéen, il se pose le problème de la *Chamaerops humilis*. Cette espèce, aujourd'hui très en retraite dans l'Europe méditerranéenne à cause de la guerre acharnée multiséculaire de l'homme, arrivait auparavant jusqu'au Sud de la France: elle jouait un rol sociologique très important, et se développait individuellement en stipe élancée. Elle aurait donc droit à une place parmi les dominates des climax. Or: est-ce qu'en égard du passé originaire devrait-on détacher en Espagne un domaine spécial caracterisé par la présence de la *Chamaerops humilis* parmi les dominantes? Ou bien, en égard de l'état présent des choses, doit-on se contenter d'établir une sous-division? Ce serait alors le cas d'employer le mot de sous-domaine.

Le problème est d'autant plus compliqué, que l'aire de la *Chamaerops humilis*, qui en dedans du domaine caractérise par la *Quercus ilex*, *Q. faginea* et *P. halapensis* ne forme qu'une bande littorale, dépasse, parallelement aux côtes la limite de ce domaine, embrassant par exemple le S. W. de la Péninsule ou la *Pinus halapensis* ne se montre plus en etat spontané. La solution demande être cherchée surtout dans l'étude géobotanique du N. de l'Afrique. Mais ce théme sort déjà hors les limites de ce travail, borne à traiter quelques questions de méthodique, pour lesquelles mon étude géobotanique de l'Espagne m'a suggéré des éclaircissements et des nouveautés.

Literature

Les ouvrages opuscules sur méthodique auxquelles se rapportent les citations et allusions contenues dans ce travail, sont si familiers aux géobotanistes, que je n'ai pas cru nécessaire en répéter une fois plus la liste. Pour la géobotanique de l'Espagne, les travaus de référence sont les suivants:

Cutanda, Vincente. Flora compendiada de Mardi dy su provincia, 1861.

Huguet-del-Villar, Emilio. Avance geobotánico sobre la pretendida Estepa Central de España, 1925 (dans la revue "Iberica," mai).

Laguna, Máximo. Memoria de reconocimiento de la Sierra del Guadarrama, 1870.

Lázaro, Blas. Compendio de la Flora Española, 2e ed., 1906.

Mazarredo, Carlos. La cuenca de abastecimiento del Canal de Isabe. II, etc., 1911.

Pascual, Augustin. Reseña Agricola, dans l'Anuario de la Comision de Estadistica del Reino, 1858.

Secall, José. Plantas vasculares de San-Lorenzo del Escorial y sus alrededores, 1889.

Willkomm, Moritz. Die Strand—und Steppengebeite der Iberischen Halbinsel und deren Vegetation, 1852.

——— Statistik der Strand—und Steppenvegetation der Iberischen Halbinsel, 1894 (1895).

——— Grundzüge der Pflanzenverbreitung auf der Iberischen Halbinsel, 1896.

PLANT COMMUNITIES OF THE JUAN FERNANDEZ ISLANDS[1]

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When I read a description of an exotic plant society I generally find that it is difficult to get a clear conception of it. Photographs of an association certainly aid in communicating to the reader a general idea of its physiognomy, but the picture thus formed in his brain lacks details in every case where he is unfamiliar with the majority of species enumerated; sometimes he does not know the genera or even the families. But the author of the description, who knows his plants, hardly considers the situation of the reader; he is quite satisfied that he has complied with the demands of science, and the scientific value of his account is perhaps just the same whether the reader knows the plants to such an extent that he becomes acquainted with the vegetation or not. Anyway, people who have a taste for comparative sociology wish the descriptions to be as enjoyable as possible. As it is, compilers who write text books on plant geography give long lists of plants characteristic of this or of that without having any idea of that of which they speak. Schimper, who was familiar with all-sorts of vegetation, endeavored to make his book more readable by means of numerous illustrations of important species, showing foliage and flowers. Others have done the same thing. Of course, this method, which is an expensive one, should be used to the greatest possible extent. It is not necessary to figure all these species; it is sufficient to illustrate all the important types, and to invent and refer to a number of expressive growth forms.

Everybody knows that several systems of life forms have been established, from Humboldt's day until ours. The last 2 were constructed by Drude and Raunkiaer. That of Drude pays too much attention to purely organographic details and cannot serve our purpose. Nor do Raunkiaer's classes express all that we would like to have expressed. This he acknowledged himself and proposed to introduce another factor than the position and protection of the buds, namely, the shape and especially the size of the leaves. All these systems were meant to be applied to the vegetation of the whole world. It might be more convenient to put off the making of such an all-comprising classification and to start with separate systems for separate regions, to use, wherever possible, well-established and generally accepted terms which are understood far and wide, and to make special types for special needs, as few as possible and as many as necessary. Among the items to be considered are the leaf characters; also the persistence

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 17, 1926.

of the foliage, the size and architecture of trees and shrubs. While it is of slight importance only, in a physiognomical sense, whether branches be opposite or alternate, such types as dichotomic trees or verticillate branches are outstanding features. Pubescence, if occurring to an extent to affect the coloring of a species, is, of course, quite worthy of notice. Regarding herbaceous plants, the branching is less important than the gathering of all the leaves in a basal rosette, the formation of mats, cushions, etc. Erect and prostrate types give a very different impression. Such a system of life forms is, of course, not at all meant to replace the classes of Raunkiaer which may be used parallel with it.

Before I come down to concrete examples I shall say a few words about my field work in Juan Fernandez, where these examples are drawn. Of late there has been a great deal of discussion both of the quadrate method and of the mode of expressing the relative importance of each species within an association. Regarding the former, I believe it is sometimes useful and sometimes not. Most of our experience was gained by using quadrates in a low and even ground stratum like a moor, an alpine heath, or the monotonous bottom cover in our northern forests. In the Juan Fernandez Islands the land surface is exceedingly broken, having become cut up into series of deep and narrow gorges, separated by very steep and narrow ridges and crests. There is no level ground and few gentle slopes. The continuous forest patches are of small extent. I tried to select squares of 10 meters to the side, and then to note all plants within. Sometimes the whole forest patch in a small valley was used as an area. It must be remembered that many species, although very characteristic of their respective stations, are rare; sometimes very rare. In order to get them in, one must use a fairly large square, or select many small ones, which takes much more time and involves considerable labor without adding materially to our knowledge. It is evident that they are not what are called constant species; they would probably be found down among the accidental ones; nevertheless, they are by no means accidental, only rare. Even if rather large units are used, it will never happen that more than one or two of the rare things are there to be put down. When it comes to the rock walls and ridges with their utterly striking vegetation it quite frequently happens that there is not room enough or no level space where even the smallest quadrate fit for use can be laid out, or rather, hung up, with the exception, of course, for the study of moss and lichen communities. I could barely select an apparently homogeneous portion and climb and scramble through it.

To indicate the frequency of each species I used the 5-graded scale of Hult-Sernander. The figures are, as everybody knows, supposed to give the area covered by each species, the summary of the projections of all individuals upon the substratum. This method has been extensively used in Scandinavia and by Scandinavians in foreign countries. When we have to do with a moss or lichen mat, or even a carpet of low-growing phanerogams where there is little or no stratification, sufficient practice will allow a keen observer to arrive at a good enough estimation. But when it comes to, let us say, a tropical forest with a multitude of tree species of all shapes and sizes, obscuring each other and woven together by climbers, I do not know how one is able to get a correct idea of the

projection of each single head, for one cannot discern the outlines of most of them. Even if we look at them from above, the result will not be satisfactory. It may well happen that 10 specimens of a species with a flat head may occupy the same space as 50 with a narrow crown; consequently, they obtain the same figure. But in the strata below there are 5 times as many trunks of one as of the other, and all may have the same average thickness, and the root system of one is perhaps 5 times as voluminous as of the other. Certainly they do not play the same physiognomic, not to speak of the biotic, rôle in that community, only in the stratum in which their crowns are located. It is perhaps necessary to count them, for we cannot be satisfied with a poor figure indicating their behavior in an upper stratum. Vines and epiphytes will generally be out of reach altogether; still, they may be important indicators of the association character. We need not, however, travel so far to find difficulties. A Swedish meadow, where 40 or 50 species of herbs and grasses are interlaced, forming a continuous succession of strata, will put our estimation power to a severe test. If we are honest we must admit that in such cases the method is personal in the extreme. It is easier if we are familiar with the species, otherwise species with an unusual and striking appearance are likely to divert us from the path of truth. All this makes me think that the 5-graded scale is almost too detailed and that in most cases we could do with three classes.

I shall now proceed to illustrate my ideas about the description of vegetation with a few examples from the Juan Fernandez Islands. Seen from our boreal point of view, their flora is exotic, and my audience is probably not acquainted with it.[2] After having looked and listened, you will tell me whether or not you have a fair idea of what the vegetation is like.

I propose to use the following roughly sketched system of life forms, where leaf sizes are added according to Raunkiaer's proposal.

GROWTH FORMS, FLOWERING PLANTS

	Form	Number of sp.	Leaf size[3]	Genus or family
I. Trees. (45 sp.)				
A. Larger (17 sp.)	Palm	1	meg.	Juania
	Magnolia	1	mes.	Drimys
	Urticoid	1	mes.	Boehmeria
	Myrtoid	7	micr.	Myrceugenia, Coprosoma, etc.
	Vaccinioid	3	micr.	Escallon, Rhaphithamnus, etc.
	Xanthoxylon	2	mes.	Fagara
	Coronilla	2	micr.	Edwardsia
B. Dwarfed (3 sp.)	Urticoid	3	mes.	Urtica, Chenopodium
C. Rosette (25 sp.)	Cordylinoid	2	mes.	Greigia, Plantago
	Palmoid	2	macr.	Dendroseris
	Brassicoid	6	macr.	Dendroseris, Nicotiana
	"	2	mes.	Dendroseris
	Dracaenoid	9	mes.	Robinsonia, Eryngium, etc.
	"	3	micr.	" " "
	Crassuloid	1	micr.	Eryngium

[2] It is not rich in species; the indigenous phanerogams are 141, the ferns 51.

[3] Under the heading "Leaf size," the following abbreviations are employed: meg. = megaphyll; mes. = mesophyll; micr. = microphyll; nan. = nanophyll; lept. = leptophyll; aph. = aphyll.

II. Shrubs. (22 sp.)				
A. Larger (6 sp.)	Bamboo	1	micr.	Chusquea
	Berberis	2	micr.	Berberis
	Vaccinioid	3	nan.	Pernettya, Ugni, etc.
B. Dwarfed. (12 sp.)	Urticoïd	1	micr.	Chenopodium
	Vaccinioid	4	micr.	Halorhagis, Wahlenbergia
	"	5	nan.	Lactoris, Wahlenb., Erigeron
	Ericoid	2	lept.	Margyricarpus, Empetrum
C. Trailing (3 sp.)	Convolvuloid	2	micr.	Rubus, Calystegia
	Vaccinioid	1	nan.	Myrteola
D. Parasitic (1 sp.)	Loranthus	1	micr.	Phrygilanthus
III. Herbs. (43 sp.)[4]				
A. Foliose erect. (16 sp.)	Various	6	mes.	Various
	"	5	micr.	"
	"	7	nan.	"
B. Arboriform (3 sp.)	Gunnera	2	meg.	Gunnera
	"	1	macr.	"
C. Rosulate (9 sp.)	Aloe	1	mes.	Ochagavia
		2	mes.	Apium, Ranunculus
		1	micr.	Plantago
		5	nan.	Compositae
D. Aphyllous succulent	Salicornia	1		Salicornia
E. Trailing (9 sp.)		1	mes.	Tetragonia
		4	micr.	Various
		4	nan.	"
F. Cushion (3 sp.)	Azorella	1	nan.	Acaena
		2	lept.	Abrotanella, Oreobanche
IV. Grasses (32 sp.)				
A. Soft tufted		22	mes.-micr.	Gramineae, Cyperaceae
B. Hard tufted	Stipa	5	mes.-micr.	Stipa, etc.
C.	Rush	4		Juncaceae, Cyperaceae
D.	Iris	1	mes.	Libertia

GROWTH FORMS, FERNS

	Form	Number of sp.	Leaf size	Genus
I. Arboreous (2 sp.)	Dicksonia	1	meg.	Dicksonia
	Cycas	1	macr.	Blechnum
II. Subarboreous (3 sp.)	Lophosoria	3	meg.	Dryopteris, Thyrsopteris, Lophosoria
III. Herbaceous (44 sp.)	Pteris	1	meg.	Pteris
	"	3	macr.	Histiopteris, Pteris
	Polypodium	5	macr.	Blechnum, Hypolepis, Polystichum
	"	8	mes.	Adiantum, Asplenium, Blechnum, Cystopteris Polypodium
	"	4	micr.	Asplenium, Polypodium
	Pellaea	1	micr.	Pellaea
	Gleichenia	3	mes.	Gleichenia
	Phyllitis	1	mes.	Elaphoglossum
	Hymenophyllum	1	macr.	Hymenophyllum
	"	7	mes.	Hymenophyllum, Trichomanes
	"	6	micr.	" "
	"	1	nan.	Serpyllopsis
	Ophioglossum	1	micr.	Ophioglossum
	Lycopodium	2	lept.	Lycopodium
IV. Herbaceous scandent (2 sp.)		2	mes.	Arthropteris, Blechnum

[4] Editorial note. It is regretted that it was impracticable to revise the tabular matter in certain sections, especially in III and IV, in accordance with the author's later suggestions. Editor.

SUMMARY

	Number	Meg.	Macr.	Mes.	Micr.	Nan.	Lept.	Aph.
Trees and shrubs	67	1	8	20	27	9	2	
Herbs	43	2	1	10	10	17	2	1
Grasses, etc.	32							
Arboreous ferns	2	1	1					
Subarbor. ferns	3	3						
Herbaceous ferns	46	1	9	21	12	1	2	
Woody climbers	wanting							
Higher epiphytes	1 Robinsonia possibly always epiphytic, Peperomia, etc., facultatively so.							

I have tabulated some of my field notes, adding the growth form and the leaf size. It is supposed that if such tables are read with due attention and are accompanied by good photographs of the association, the reader will be able to get a fairly accurate idea of the vegetation.

Stratification is indicated according to the Swedish system. This answers fairly well to conditions in Juan Fernandez, but of course we are quite at liberty to make other subdivisions to suit other types of vegetation.

The observations on the character of the station, the soil, etc., are omitted here; also the lists of lower cryptogams.

MASATIERRA. SUBMONTANE, DRY EVERGREEN FOREST
(250 m.)

Growth form	Leaf size	Names of species	Value
		I	
Myrtoid	micr.	Myrceugenia fernandeziana	5
Xanthoxylon	mes.	Fagara mayu	1
		II	
		Myrceugenia	4
		Fagara	1
		IV	
		Myrceugenia	3
Pteris	macr.	Pteris Berteroana	1
Lophosoria	meg.	Dryopteris inaequalifolia	1
		V	
Soft grass		Uncinia Douglasii	1
Polypodium	macr.	Polystichum vestitum	1
		VI	
Polypodium	mes.	Blechnum valdiviense	2
		VII	
		Dry leaves, few cryptograms on ground, several on stumps and trunks	

MASATIERRA. SUBMONTANE EVERGREEN FOREST
(435 m.)

Growth form	Leaf size	Names of species	Value
		I	
Pistacia	mes.	Fagara mayu	1
		II	
Myrtoid	micr.	Myrceugenia fernandeziana	4
Vaccinioid	micr.	Rhaphithamnus venustus	2
Myrtoid	micr.	Coprosma pyrifolium	1
"	micr.	" triflorum	1
Magnolia	mes.	Drimys Winteri var.	1
Brassicoid	mes.	Dendroseris micrantha	1
Palm	meg.	Juania australis	1 (one sp'm.)

Growth form	leaf size	Names of speices	Value
		III	
		Myrceugenia	4
		Drimys	1
		Rhaphithamnus	1
Gunnera	meg.	Gunnera peltata	1
Lophosoria	meg.	Lophosoria quadripinnata	1
		IV	
Polypodium	macr.	Polystichum Berterianum	3
Dicksonia	meg.	Dicksonia Berteroana	1 (rare)
		V	
		Polystichum	3
Polypodium	macr.	Blechnum chilense	2 (greg.)
Iris	mes.	Libertia formosa	1
Polypodium	mes.	Adiantum chilense	1
Soft grass		Uncinia Douglasii	1 (greg.)
		VI	
Trail'g. herb	micr.	Dysopsis glechomoides	3
		VII	
		Dry leaves, moss patches.	
		Several epiphytic ferns, mes. and micr.	

MASATIERRA. MONTANE EVERGREEN FOREST
(510 m.)

Growth form	Leaf size	Name of species	Value
		II	
Myrtoid	micr.	Myrceugenia fernandeziana	2
Magnolia	mes.	Drimys Winteri var.	2
Dicksonia	meg.	Dicksonia Berteroana	2
Vaccinioid	micr.	Azara fernandeziana	1
"	micr.	Rhaphithamnus venustus	1
Myrtoid	micr.	Coprosma pyrifolium	1
Brassicoid	mes.	Dendroseris micrantha	1
Dracaenoid	mes.	Robinsonia evenia, epiphyte on Dendroseris	1
		III	
Vaccinioid	micr.	Escallonia Calcottiae	1
Myrtoid	micr.	Coprosma triflorum	1
"	micr.	Cuminia eriantha	1
Dracaenoid	mes.	Rhetinodendron Berterii	1
"	mes.	Robinsonia thurifera	1
Berberis	micr.	Berberis corymbosa	1
Vaccinioid	nan.	Pernettya rigida	1
Gunnera	meg.	Gunnera peltata	1
Scandent fern	macr.	Blechnum Schottii	1
		IV	
Lophosoria	meg.	Dryopteris inaequalifolia	2
"	meg.	Thyrsopteris elegans	2
Myrtoid	micr.	Myrceugenia fernandeziana	1
Vaccinioid	nan.	Lactoris fernandeziana	1
Gunnera	meg.	Gunnera peltata	1
Cycas	macr.	Blechnum cycadifolium	1
Lophosoria	meg.	Lophosoria quadripinnata	1
Pteris	macr.	Pteris Berteroana	1
		V	
Polypodium	macr.	Blechnum chilense	3
Scandent fern	macr.	" Schottii	3
Polypodium	macr.	Polystichum Berterianum	2 (greg.)
Vaccinoid	micr.	Halorrhagis masatierrana	1
Pteris	macr.	Histiopteris incisa	1
Foliose herb	micr.	Acaena ovalifolia	1
Hymenophyllum	macr.	Hymenophyllum fuciforme	1

Growth form	Leaf size	Name of species	Value
		VI	
Trail'g. herb	micr.	Dysopsis glechomoides	2
Polypodium	micr.	Blechnum valdiviense	1
Hymenophyllum	micr.	Hymenophyllum dichotomum	1
		VII	
		Cryptogams	
		Numerous small ferns as epiphytes	

MASATIERRA. MONTANE DICKSONIA FOREST
(660 m.)

Growth form	Leaf size	Names of species	Value
		I	
Magnolia	mes.	Drimys Winteri var.	1
		II	
		Drimys	1
Myrtoid	micr.	Coprosma triflorum	1
		III	
Myrtoid	micr.	Cuminia eriantha	1
"	micr.	Myrceugenia fernandeziana	7
Dicksonia	meg.	Dicksonia Berteroana	4
Lophosoria	meg.	Thyrsopteris elegans	2
Cycas	macr.	Blechnum cycadifolium	1 (rare)
Palmoid	macr.	Dendroseris pinnata	1
Dracaenoid	mes.	Robinsonia evenia epiphyte on Dicksonia	1
		IV	
Cycas	macr.	Blechnum cycadifolium	2
Gunnera	meg.	Gunnera peltata	1
Lophosoria	meg.	Dryopteris inaequalifolia	1
Polypodium	macr.	Blechnum chilense	1
		V	
Polypodium	macr.	Polystichum Berterianum	1
"	macr.	" vestitum	1
		VI	
Polypodium	mes.	Blechnum valdiviense	2
"	mes.	Asplenium macrosorum	1
		VII	
		Few cryptogams.	
		Climbing as high as to stratum III: Blechnum Schottii	3
		Several smaller ferns epiphytic on Dicksonia and on Drimys	

MASATIERRA. EVERGREEN BRUSHWOOD ON ROCKY RIDGE
(550 m.)

Growth form	Leaf size	Names of species	Value
		III	
Magnolia	mes.	Drimys Winteri var.	1
Myrtoid	micr.	Coprosma triflorum	1
Rosette tree	mes.	Robinsonia thurifera	1
		IV	
Vaccinioid	nan.	Ugni Selkirkii	4
"	nan.	Pernettya rigida	3
Cycas	macr.	Blechnum cycadifolium	3
Gunnera	meg.	Gunnera peltata	1
Vaccinioid	micr.	Coprosma triflorum	1
Myrtoid	micr.	Escallonia Calcottiae	1
Palmoid	macr.	Dendroseris pinnata	1
Brassicoid	mes.	" micrantha	1
"	macr.	" marginata	1
Dracaenoid	mes.	Centaurodendron dracaenoides	1
"	mes.	Robinsonia Gayana	1
"	micr.	" gracilis	1

Growth form	Leaf size	Names of species	Value
		V	
Gunnera	meg.	Gunnera bracteata	2
Dracaenoid	mes.	Dendroseris micrantha	1
Polypodium	macr.	Blechnum chilense	2
"	macr.	Polystichum Berterianum	2
Vaccinioid	micr.	Halorrhagis masatierrana	1
Polypodium	mes.	Blechnum Schottii	1
Soft grass		Bromus fernandezianus	1
		VI	
Hymenophyllum	mes.	Hymenoglossum cruentum	1
"	micr.	Hymenophyllum dichotomum	1
		VII	
		Numerous cryptograms	

MASATIERRA. STEPPE IN THE DRY BASAL REGION

Growth form	Names of species	Value
	IV	
Stipa	Stipa fernandeziana	2
Soft grass	Polypogon chilensis	1
"	Carex Berteroniana	1
Rush	Scirpus nodosus	1
	V	
Stipa	Piptochaetium laevissimum	5
Soft grass	Bromus unioloides (a weed)	2
"	Setaria imberbis (a weed)	1
"	Uncinia Douglasii	1
	VII	
	Some mosses and lichens	

MASAFUERA. ALPINE HEATH (1350–1400 m.)

Growth form	Leaf size	Names of species	Value
		A.—V	
Vaccinioid	nan.	Pernettya rigida	1
Soft grass		Anthoxanthum odoratum (a weed)	3
" "		Carex Banksii	1
		VI–VII	
Loose cushion	micr.	Acaena masafuerana	5
Soft grass		Uncinia tenuis	2
Trail'g shrub	micr.	Rubus geoides	1
Trail'g herb	nan.	Nertera depressa	1
		Cryptogams important	
		B.—V	
Vaccinioid	nan.	Pernettya rigida	1
Soft grass		Anthoxanthum odoratum (a weed)	3
Lophosoria	meg.	Lophosoria quadripinnata	1
Soft grass		Agrostis masafuerana	1
Iridoid	mes.	Libertia formosa	1
		VI–VII	
Loose cushion	micr.	Acaena masafuerana	2
Soft grass		Luzula insularis	1
Aster	micr.	Erigeron turricola	1
Gnaphalium	micr.	Gnaphalium spiciforme	1
Aster	nan.	Lagenophora Harioti	1
		Cryptogams	
		C.—V	
Gleichenia	mes.	Gleichenia quadripartita	2
Polypodium	macr.	Blechnum chilense	1

Growth form	Leaf size	Names of species	Value
		VI–VII	
Trail'g herb	nan.	Nertera depressa	3
Lycopodium	lept.	Lycopodium Gayanum	2
Trail'g shrub	micr.	Rubus geoides	1
Trail'g herb	lept.	Galium masafueranum	1
Rosette herb	nan.	Lagenophora Harioti	1
Hymenophyllum	micr.	Hymenophyllum falklandicum	1
"	nan.	Serpyllopsis caespitosa	1
		Cryptogams important	

It should be stated that this method is all right when we have to deal with a poor and easily-handled flora like that of Juan Fernandez, but that it is bound to fail when it comes to associations with hundreds of species. As far as I know, not one description in a modern sense of a rich tropical association exists. Possibly such detailed descriptions are not necessary; we may be entitled to leave out many of the components and still be able to separate the associations from each other. This is perhaps what we ought to find out first of all.

Finally, I shall ask you to bear with me a little longer, as I should like to make a few concluding remarks on the part taken by the various elements of the flora in the associations I have mentioned. This insular flora is, as everybody knows, highly peculiar; by this I understand that it is very unlike the Chilean in spite of the comparatively small distance between them, barely 360 miles. Seventy per cent of the phanerogams and 33 per cent of the ferns are endemic, with many endemic and well-characterized genera and quite peculiar species in most of the non-endemic genera. The genetic elements are neotropic-andine, paleotropic-monsoonian (including Australian types), Antarctic, Paleopacific, and ubiquitous, the latter very insignificant. These elements are not evenly distributed through associations and regions. The treeless basal region and lower forests are mainly neotropic and contain very few remarkable endemics. The Paleopacific rosette-trees of several families are found, together with a number of other peculiar and systematically isolated types in the montane region, with its greater rainfall and frequent fogs. On the whole, the same holds true for the Hawaiian vegetation. Of the Antarctic element, most of the species belong to a group that inhabits the Magellanic and Subantarctic lands. In Juan Fernandez this group is confined to the summit plateau of Masafuera. Suitable stations are lacking in the other island, which is much less elevated. We do not find Magellanic species only, but we find Magellanic associations reproduced on a small scale. It is not difficult to trace this alpine vegetation back to its origin; it has the character of an outpost from a flora that still occupies vast areas in the far south. The Andine flora in Juan Fernandez is also nothing but a poor reproduction of the strong and varied flora that inhabits the flanks of the great Andean chain. The Paleopacific and Paleotropic element is another story. It is not found in Chile. In Juan Fernandez it shows a most decided accumulation in the montane region and puts its stamp on the whole flora and vegetation there. Members of the same element are scattered in the Pacific, with a stronghold in Hawaii, where I have seen a good deal of the flora and studied its mode of

occurrence. The Paleopacific flora either lacks or shows only vague relations to the floras of the surrounding continents. Nothing has hitherto been able to convince me that it has sprung in so many places from so many roots. It is difficult to prove anything in a case like this, but it is logical to suppose that the rosette tree associations in Juan Fernandez are, as it were, relict associations, fragments which have survived and which barely manage to get along. Their flora is distinct from both the Andine and the Antarctic, which are still flourishing, while the Paleopacific is scattered far and wide, because it has lost the ground under its feet. With few exceptions, all the isolated endemic plants in Juan Fernandez are rare, sometimes so scarce that it would be an easy matter, provided we were able to reach some of their last and very inaccessible stations, to exterminate them. They do not even move from one of the islands to the other; few of them are common to both. All talk of an oversea migration under the present conditions is useless and only apt to divert us from our efforts to trace this scattered flora back to its common origin.

THE VEGETATION OF THE FOREST FLOOR; LIGHT VERSUS SOIL MOISTURE[1]

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One of the most characteristic features of forest vegetation is the great variation in the volume and character of the growth that originates, and persists beneath the canopy of the uppermost layer. Some stands of timber have a dense jungle of vegetation beneath the uppermost layer while others of the same density are free, or nearly free, of vegetation on the forest floor. The structure of forest vegetation as made up of various layers has been explained as controlled by light. The assumption is that the more light-needing elements in the vegetation form the upper layer and each of the subordinate layers is progressively less light-demanding.

Much of the theory of silvicultural practice has been built around this assumption, thus in selection cuttings, in shelterwood cuttings, and in group cuttings, current literature states that the reproduction which follows the cutting is due to the increased intensity of the light which penetrates the canopy and reaches the forest floor. When a tree falls in the virgin forest the opening made in the canopy permits more light to reach the ground beneath the opening, and, as a consequence, germination takes place and soon a dense reproduction is in evidence.

An important paper by Heyer was published in 1852 under the title "Verhalten der Waldbäume gegen Licht and Schatten." The theory of silvicultural practice and its relation to light and shade expounded by Heyer almost immediately took possession of the forestry profession and has been a basic guide in silvicultural practice ever since. The theory that light intensity on the forest floor controls reproduction has been much over-stressed. It is only beginning to be appreciated that factors other than light are often of equal or even greater importance in determining the origin, growth, and development of vegetation forming the subordinate layers beneath the canopy.

An article by Fricke published in 1904 under the title "Licht- und Schattenholzarten ein wissenshaftlich nicht begründetes Dogma" controverts the over-emphasis placed on light as the controlling factor in reproduction under forest canopies. Fricke's experiments show that deficient soil moisture often prevents germination and causes the death of young growth under the shelter of mother trees, and that soil moisture rather than light often controls the presence of

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 18, 1926.

reproduction and other vegetation under forest canopies. His experiments were conducted in a 100-year old Scotch pine stand. A number of small plots were established under the canopy. Each of these plots was trenched in the spring in order to cut off all roots from adjacent trees which extended into the trenched plots. An equal number of untrenched plots was established at the same time. All plots were then sown with several species of tree seeds without previous preparation of the soil. An excellent reproduction was obtained on the trenched plots with little or none on the check plots, although under canopies of equal densities. The soil moisture in the trenched and check plots was determined at intervals during the growing season. It was found that the soil moisture in the trenched plots was 2 or more times as much as in the check plots during the driest periods of the summer, and that the difference increased with the progress of the season. Fricke also made trenches so as to isolate plots of young growth under old stands of pine and found that the trenching had a marked effect on the vigor and development of the young growth. He concluded from his experiments that the failure or decline of reproduction under old stands was due largely to root competition for soil moisture and not primarily to the shade effect of the overwood. He states that the so-called "light increment" or increase in growth following thinnings is not only due to increased light, but is very largely due to decreased root competition for available soil moisture.

These experiments by Fricke have not been given the attention by foresters and ecologists that they merit. We still look upon light as the controlling factor in the germination and early growth of tree seedlings under forest canopies. We explain the abundant reproduction and the vigor and rapidity of growth of the seedlings that appear in openings in the stand where trees have fallen or have been cut down, as due to increased light. We explain the absence of shrubs and herbaceous vegetation on the forest floor as due to insufficient light.

Five years ago the writer of this paper undertook an extensive series of field experiments on the Yale Demonstration and Research Forest at Keene, New Hampshire, in which some of the earlier experiments by Fricke were repeated and additional investigations undertaken in a study of germination and early growth under canopies of different densities, where root competition for soil moisture was under control. This paper is a report on the results of these studies. It relates to (a) the effect of the high, fully-stocked, forest on available soil moisture over the growing season and (b) the effect of the withdrawal of root competition on the origin, survival, and growth of the lesser vegetation beneath canopies.

That part of the forest where the soil moisture studies were conducted is nearly level, forming a part of a broad valley. The soil is of sedimentary origin and has a reaction varying from pH 5.6 in the top layer to 6.0 at a depth of 3 feet. The soil is coarse sand, very uniform in texture, and originally bore a vegetation in which white pine was dominant and often in pure stands. The soil moisture studies extended over a period of 2 years during which the soil moisture per unit volume of soil was determined in each successive 4 inch layer of soil to a depth of 3 feet. Samples were taken at intervals throughout the year but mostly during the driest periods of the growing season. These samples were each 1000 ccm.

in volume of soil in place. They were taken in the open on a site denuded of all vegetation and also under a nearby fully-stocked stand of 60 - 80 year old white pine growing at the same elevation on the same type of soil. Special attention was directed to having these 2 sites as nearly alike as possible with the exception of the vegetation.

Mechanical analyses of composite samples carefully selected and at each successive 4 inches of soil-depth down to 3 feet on each site showed great uniformity with the exception of greater humus content in the surface layers from the forested site. Not only was the physical water content per given volume of soil in place determined, but also the wilting coefficient at each depth in order to ascertain the actual available water over the growing season. The results of these studies, which are as yet unpublished, very clearly show that the forest progressively exhausts the available water in the soil with the progress of the growing season and that the forest soil with its normal layer of litter has the greatest amount of moisture after mid-summer in the upper 4-inch layer even during the driest periods, and the amount of water in each successive layer progressively decreases with soil depth down to at least 3 feet. This apparently explains why very tolerant species like eastern hemlock and flowering dogwood, that germinate and persist under dense canopies, have shallow initial root systems.

On the other hand, the denuded soil, where samples were taken also at each depth down to 3 feet, remained on the whole much moister than the forested soil, as shown in samples from corresponding depths in the forest station. Even the uppermost soil layer of 4 inches in the denuded area contained, after midsummer, as much or even more available moisture at critical periods than the surface layer beneath the litter in the forest. It appears that both the denuded soil in the open and the soil under the forest canopy are saturated with water after the melting of the snows in spring. The water that remained in the soil, after the excess was carried away by gravity, was progressively reduced on the denuded site by evaporation from the soil surface but was replenished at irregular intervals by precipitation. Although the loss due to evaporation was rapid, so long as the surface soil remained moist, it was slow as soon as the surface soil became dry, and served as a dust mulch. Due to the slight loss of moisture from the exposed soil after the top layer became dry, and the replenishing of the soil moisture by precipitation, each successive layer of the soil on the denuded site down to a depth of 3 feet had available water in considerable quantity, even during the driest periods of the growing season. On the other hand, although the soil on the forested site was equally saturated after the melting of the snow in spring, and the loss through surface evaporation was reduced through the effect of the surface litter, precipitation was much less effective in replenishing the soil moisture supply, due to the withholding of a part of it by the canopy and a part also by the litter of the forest floor. Most of the rains that fell during the growing season did not add essentially to the water supply of the forest soil, as it was taken up by the canopy and litter and later returned to the atmosphere through evaporation. The heavier precipitation which penetrated the denuded

soil in the open to a depth of 3 feet seldom penetrated to a depth of 6 inches in the forest.

The striking characteristic of these soil moisture determinations is the extent to which a fully stocked 60–80 year old stand of white pine progressively depletes the soil moisture through transpiration loss with the progress of the growing season. The average available water content of the upper 3 feet of the forest soil, after mid-summer, was found to be one-half to one-third of that of the bare soil in the open. The extent to which the fully-stocked high pine forest depletes the soil moisture is clearly brought out in the following experiments in which reproduction and lesser vegetation on small quadrats were relieved from root competition from the surrounding trees by trenching, so as to cut off all roots from the outside that extended into the trenched areas.

Fig. 1. Sample Plot No. 1. View on east side of plot showing the height and density of the grey birch overwood. Picture taken from an open space a few feet from the border of the plot. July, 1922.

The trenching experiments were begun in July 1922 and were of 2 general types: (a) Trenching around white pines planted in 1917 under a dense overwood of grey birch, (b) trenching around small quadrats rectangular in form 9 and 6 feet on a side located beneath stands of white pine of different ages.

In July, 1922, a plot one-fourth acre in area was located on a 10° slope having a western aspect (Fig. 1). The corners were permanently marked with iron pipes driven 2 feet in the ground. The trees forming the upper story were sufficiently dense to form a complete canopy and were almost entirely grey birch. The vegetation beneath the superior layer (Fig. 2) consisted of a scattered growth of low herbaceous vegetation, a few shrubs, and the planted red and white pine.

In Table 1 the trees forming the upper story are classified into 1-inch diameter classes, and it shows the number of trees of each diameter class on the plot

TABLE 1 THE DENSITY OF OVERWOOD

	Number of stems on plot	Number of stems on acre	Diameter in inches
Grey birch	640	5926	0–1
	310	2870	1–2
	100	926	2–3
	1	9	3–4
White pine	3	27	0–1
	1	9	1–2
	0	..	2–3
	0	..	3–4
	1	9	4–5

Fig. 2. Sample Plot No. 1. Camera set up at corner A looking diagonally into the plot. The view shows the homogeneous character of the grey birch overwood. July, 1922.

together with the data calculated on an acre basis. The great density of the upper layer is shown in that the stand consisted of 9,776 trees per acre, varying from 1 to 5 inches in diameter, and formed a canopy some 12–18 feet above the ground. The ground cover on the plot was composed of 47 species of herbaceous plants and low shrubs. Forty-eight per cent of the area was bare of surface vegetation.

The coarse sandy soil contained from 39 to 57 per cent of coarse and fine gravel. The silt and clay were less than 10 per cent of the total dry weight of the soil. The organic matter in the first foot of soil varied from 6.2 to 10.1 per cent of the total dry weight. At a depth of 2 feet it varied from 1.7 to 4.1 per cent. The surface soil was decidedly acid, reacting pH 5.5. At lower depths it varied

from neutral to 6.5. Due to the nature of the soil, the degree of slope, and the aspect, the site might be classed as relatively dry for the region.

The average height of the seedlings when planted in May 1917 was 3.5 inches. In July, 1922, all the planted pines on the plot were permanently marked with metal tags bearing serial numbers. They were also measured and the annual height growth of each tree recorded. Seventy-five trees were tagged and measured, of which number 20 were trenched and the remainder were left untrenched. These pines varied from 9.5 to 29 inches in height when measured and the average height was 19.4 inches. The average height of the trenched trees was but 14.5 inches. Thus it may be seen that before trenching the average annual rate of growth in the trenched trees was less than in the untrenched trees left as checks.

The trenching was done with a sharp spade inserted vertically in the soil in a circle around each tree (Fig. 3), the circle having a diameter of 3 feet with

Fig. 3. Sample Plot No. 1. This view shows White Pine No. 55, the trench surrounding it, and the ground cover. July, 1922.

the tree in the center. All roots of adjacent trees that entered the trenched areas were thus severed in the first foot of soil. The litter and surface soil were undisturbed. Annual measurements of height growth were made both on the trenched and untrenched trees over a period of 4 years, the trees being retrenched annually. The year following the first trenching, the 20 trenched trees increased their annual height growth 45 per cent over the average annual growth of the 4 years preceding trenching. Comparing the average height growth of the untrenched trees with the average growth of the trenched trees for the same year, it appears that there is a difference of 14 per cent in favor of the latter, although

the trenched trees were growing slower than the untrenched trees before trenching. The trees were retrenched in 1923, 1924, and 1925. Measurements made each year showed an accelerated growth in the trenched trees as compared with the growth in the same year in the untrenched trees.

The increase in the average height growth for the 3 years subsequent to the trenching over that for the 5 year period preceding the operation was 337 per cent greater for the trenched than for the untrenched trees.

A study was made in midsummer 1922 of the average photochemical light intensity that the planted trees were subjected to as compared with full light intensity at the same times. Even under this extremely dense overwood of nearly 10,000 trees per acre, 1–5 inches in diameter, the photochemical light intensity 3 feet above the surface, taken with a photometer, varied at 12 M on days having full light intensity from an average of 0.114 to 0.13 of full sunlight. It is very significant that a stand of grey birch of this density permits from approximately one-eighth to one-ninth of the total photochemical light intensity that reaches the canopy to pass through to the forest floor. This is believed to be largely due to the light color of the foliage, bole, and branches of the grey birch.

It appears from these studies that the death or slowing down in the growth in white and red pine growing under dense stands of grey birch is primarily a reaction to deficient soil moisture at critical times during the growing season. The reduction of light intensity by the birch canopy appears entirely inadequate to explain the retardation of growth in the pine beneath.

The reduction in the available water supply in the forest soil by a dense overwood is strikingly brought out in the following experiment in which selected quadrats were established beneath stands of white pine varying in age from 25 to 60 years. In each set of experiments, 1 or more quadrats 6 and 9 feet on a side were trenched and an equal number that were otherwise similar were left untrenched to serve as checks.

In July, 1922, a trenched quadrat 9×9 feet was established beneath the canopy of a 40–60 year old stand of white pine where the ground cover was chiefly pine needles, although there was a scanty surface vegetation of herbaceous plants and moss and a scattered reproduction of pine and hemlock 2 years old. The trenched and the check quadrats were located and charted with reference to the surrounding trees. Although the trenched quadrat was 9×9 feet the 2 untrenched quadrats that served as checks were 4.5×9 feet, one at each side of the trenched quadrat. The area on which the quadrats were established was surrounded by a low wire fence.

The trench around the quadrat was excavated to a width of 1 foot and a depth of 3 feet (Fig. 4). The location of each pine root passing into the trenched area was charted (Pl. I, Fig. 1). All roots were cut off along each wall of the trench. The trench was then refilled and the vegetation of the quadrat photographed (Fig. 5). A total of 15 species of low shrubs, herbaceous plants, moss, and 2 year old tree seedlings were present on the trenched quadrat (Pl. I, Fig. 2). Twenty-five white pine, 1 pitch pine, and 9 hemlock from 1 to 2 inches in height

were found and located on the chart. The distribution and character of the vegetation of the untrenched check quadrats were practically the same as on the trenched, with the exception that the average density was 24 per cent greater. The total number of species present was the same and they occupied the same relative position as to abundance.

Five composite soil samples were taken from the soil profile exposed in trenching. Each sample was made up of 4 parts from near the 4 corners of the

Fig. 4. Sample Plot No. 5. A view of the trenched quadrat showing the depth of the trench and the exposed roots in the trench walls. August, 1922.

Fig. 5. Sample Plot No. 5. A view showing the quadrat, after trenching, strung at foot intervals for charting the vegetation. August, 1922.

trenched quadrat. The light, yellow coarse sand was relatively free from stones and was easily excavated. Mechanical analysis gave from 8 to 21 per cent of coarse gravel and 11–14 per cent of fine gravel; more than 50 per cent of all samples was coarse and medium sand; 4–11 per cent was silt and clay. The organic matter determined by ignition varied from 7 per cent by weight in the surface sample to 1.1 per cent in the sample from the greatest depth. The surface samples gave a pH value of 6.5.

A comparison of the average photochemical light intensity over the quadrats with full light intensity at the same time made from 10 A.M. to 2 P.M. on clear days in midsummer gave in 13 tests the chemical light intensity 3 feet above the forest floor at one-fourteenth that of full sunlight.

Fig. 6. Sample Plot No. 5. A view showing the contrast in the character and vigor of the vegetation inside and outside the trenched quadrat 2 years after trenching. July, 1924.

Soil samples taken from a depth of 0–6 inches in the trenched quadrat on July 13 and again on September 8, 1923, and an equal number taken at depths of 6–12 inches were compared with similar samples taken from the check plots.

On July 13, 1923, the average moisture in the first 6 inches of soil of the untrenched plot was 6.3 per cent and of the second 6 inches 8.1 per cent of the total dry weight. On the same date the average moisture in the trenched area at the same depths was respectively 16.4 and 15.4 per cent.

On September 8, 1923, when samples were again taken the untrenched plot showed 11 per cent of moisture in the first 6 inches and 9.3 per cent in the second, while on the trenched plot there was 15.4 per cent in the first 6 inches and 18.6 per cent in the second 6 inches.

The above samples were taken during the driest periods of the growing season. Similar samples were taken during dry periods in 1924 and 1925 with essentially the same results. From the numerous soil moisture tests made over a period of

3 years on the above plots it appears that trenching so as to cut off the roots of adjacent dominant vegetation from entering from outside increased the physical soil moisture of the first foot during critical periods from an average of 9.5 to an average of 16.4 per cent. Even more important than the increase in physical water content was the increase in available water.

The contrast between the vigor and growth of the surface vegetation on the trenched quadrat as compared with that on the untrenched quadrats began to be apparent a few months after trenching. In September, 1923, the year following trenching, after a dry August, the vegetation on the trenched quadrat was still fresh and thrifty, while that on the untrenched plots appeared to be in poor condition from lack of available soil moisture. In early September of the following year, that is, the beginning of the second year after trenching, the contrast in the surface vegetation in the trenched with the untrenched area was even greater (Fig. 6). Soil samples taken at this time gave an average water content in the upper foot of soil in the untrenched area of 7.6 and in the trenched area of 21.4 per cent. In late August, 1925, more than 3 years after the first trenching,

Fig. 7. Sample Plot No. 5. A view showing the contrast in the character and vigor of the vegetation inside and outside the trenched quadrat 3 years after trenching. July 1925.

the trenching being repeated at 2 year intervals, the surface vegetation on the trenched plot was carefully compared with that on the untrenched plots. The trenched plot was also recharted. Due to the marked increase in soil moisture in the trenched plot a succession had occurred, and some of the species most abundant when the plots were established had disappeared or become greatly reduced in numbers, while other species, some of which were entirely absent when the plots were established, were now abundant on the trenched area (Fig 7). It is interesting to note that the number of moist soil and swamp species which were entirely absent when the plot was first trenched now appeared in the vegetation. An analysis of the vegetation on the trenched plot (Pl. I, Fig. 3)

showed the presence of 31 species in contrast with 15 when the plots were established, while on the untrenched quadrats the original 15 species had been reduced to 7. The more xerophytic species persisted, while on the trenched plot some xerophytic species had been replaced by balsam (*Abies balsamea*) and other species characteristic of moist surface soils. The fairly abundant reproduction of 2 year old seedlings of pine and hemlock present on the plot when established had on the untrenched plots been reduced to 5 per cent of their original number. The total number of tree seedlings now on the trenched plot was 21 pine, 12 hemlock, 1 balsam, 2 grey birch and 4 aspen, all of which were making fairly vigorous growth. There was on the whole a considerable increase in the tree seedlings on the trenched plot 4 years after trenching. The grey birch and the aspen showed no indications of suffering from lack of light.

Fig. 8. Sample Plot No. 10. View of the trenched and untrenched quadrats from the north, the trenched quadrat at the right. Quadrats established and trenched in September 1923.

Additional trenched and check plots were established in 1923 and 1924. These plots have given results to date similar to those already described. In general, the effect of the trenching has been to greatly increase the physical water content of the soil on the trenched areas during the driest periods of the growing season. This increase in the trenched plots was often as much as from 100 to 200 per cent, and in some cases even more over that of the check plots, freely penetrated by the roots of the trees forming the upper canopy. The vegetation of the trenched and check plots, even under the densest stands of white pine, is already strikingly different.

In September (1923) trenched and untrenched plots, each 6 feet on a side, were established under a very dense 30-year old pure stand of white pine (Fig. 8), The stand of pine where these plots were established was so dense there was no vegetation whatsoever on the forest floor (Fig. 9), the ground being covered with

a uniform layer of pine needles from 1.5 to 2.5 inches in thickness. The quadrat at the right, shown in Figure 8, was trenched in the same manner as already described. The position of the roots of the surrounding trees that were severed in the trenching were charted and tabulated as to size, the method being shown in Plate I, figure 1. It is to be noted that nearly all of the pine roots from the surrounding trees were in the upper foot of soil, very few reaching a greater depth than 3 feet. Four soil samples were taken at various depths and their reaction and texture determined. The surface layer had a pH value of 6.0 which increased to 6.5 at 3 feet. Mechanical analysis showed from 20–30 per cent of coarse and fine gravel and 9 to 16 per cent of silt and clay, the remainder being fine gravel, coarse and medium sand. The photochemical light intensity over the plots as compared with that of full light at the same time averaged for the tests made but one sixty-fourth of full light.

Fig. 9. Sample Plot No. 10. Vertical view showing the layer of pine needles on the quadrats at the time of trenching and the absence of vegetation. September, 1923.

In April (1924) the leaf litter was removed from one-half of each quadrat and 1 ounce of white pine seed, having a viability of 84 per cent, was uniformly sown over each, both on the litter and on the part where the litter was removed. Each quadrat was divided into squares 1 foot on a side and a record kept of the vegetation that appeared on each square foot of surface.

A count of the pine seedlings was made at fortnightly intervals during the growing season after July 2, 1924, when the first count was made. On the trenched plot with the litter removed there was on September 10, an average stand of 56 pine seedlings per square foot (Fig. 10). The average stand on the part of the same plot with the litter intact was only 1.5 seedlings per square foot. On the untrenched plot there was on the part with the litter removed 3 seedlings

per square foot and on the part with the litter intact one-eighth seedling. Going along with this remarkable contrast in germination and early survival on the trenched as compared with the untrenched quadrats was an equally significant contrast in soil moisture. Duplicate samples of the first foot of soil from the trenched and untrenched plots gave the average moisture content during the driest periods of 1924 and 1925 as shown in the following table.

TABLE 2. MOISTURE CONTENT IN THE UPPER FOOT OF SOIL ON THE TRENCHED AND UNTRENCHED PLOTS DESCRIBED IN THE TEXT, AT THE DRIEST PERIODS IN 1924 AND 1925

Date	Quadrat	Moisture content, depth 0-6 inches		Moisture content, depth 6-12 inches		Average moisture content, 0-12 inches	
		Litter removed	Litter intact	Litter removed	Litter intact	Litter removed	Litter intact
1924							
July 8	Trenched	25.7	23.5	23.01	15.1	24.8	20.0
" 8	Untrenched	9.5	8.98	9.1	9.	8.7	8.9
July 25	Trenched	33.5	28.8	25.1	26.6	31.1	25.8
" 25	Untrenched	9.6	10.5	9.0	8.9	10.0	9.0
1925							
July 30	Trenched	26.9	27.7			27.3	
" 30	Untrenched	8.5	8.8	7.9	8.6	8.7	8.3
August 14	Trenched	27.5	27.6	21.4	22.8	27.5	22.1
" 14	Untrenched	15.5	14.3	8.4	6.2	14.9	7.3

This table clearly shows the great influence of this 30-year old fully stocked stand of white pine in reducing the water content of the soil. These results are in full accord with those from other plots presented in the earlier part of this paper.

Fig. 10. Sample Plot No. 10. View of the trenched quadrat showing the part where the litter was removed at the right. Note the reproduction of white pine (64 to the square foot) on the part of the quadrat where the litter was removed and the almost complete absence of seedlings on the part with the litter intact. July, 1924.

A fall of pine needles to a depth of one-half inch was added to the litter in September and October (1924). The following spring (1925) when the plots were examined in May the abundant reproduction on the trenched plot where the litter was removed when seeded the previous year had been smothered under the fresh litter and the heavy snows of the preceding winter.

In July (1925) when a count was made, the seedling pines on the part of the trenched quadrat from which the litter was removed had been reduced from 56 per square foot to 3.2, and where the litter was not removed, from 1.5 to 0.6. On the untrenched quadrat 2 seedlings from the previous year's germination were found on that part of the 8 sq. ft. having the leaf litter removed, and only 1 on the part of the 8 sq. ft. with the litter intact.

The coming in of an abundant and varied natural vegetation on the trenched quadrat was observed when the counts were made, while there was little natural vegetation on the check quadrat. When these quadrats were recharted in August, 1926, nearly 3 years after their establishment, there were 20 species of herbaceous and woody plants on 8 square feet of that part of the trenched quadrat from which the litter had been removed, and but 7 on the similarly treated part of the untrenched quadrat. Of greater significance, however, was the difference in the number of individuals per square foot, namely 32 on the former and but 4 on the latter (Fig. 11). On the other hand, that part of the

Fig. 11. Sample Plot No. 10. View of the trenched quadrat showing the part where the litter was removed in the foreground. Note the abundant volunteer growth. August 1925.

trenched quadrat with the litter intact had but 4 plants per square foot and the similar part of the untrenched plot had only 1 plant per square foot.

The migration of vegetation into the trenched area particularly on the part where the litter was removed (Fig. 11) as compared with the untrenched area (Fig. 12) is most significant and indicates at least that the factor controlling the entrance of natural vegetation under a forest canopy is not light under the conditions of these experiments. The 2 controlling factors appear to be soil moisture and leaf litter. The larger consumption of water by the stand of pine so reduced the moisture content of the soil during periods of drought the lesser

Fig. 12. Sample Plot No. 10. View of the untrenched quadrat showing the part where the litter was removed at the right. Note the absence of volunteer growth. August, 1925.

plants were forced out or were unable to enter and become established. The depth of leaf litter is so great it inhibits germination and early growth. Even when germination occurs on areas from which the litter has been removed the young seedlings are mostly smothered beneath the leaves that fall the autumn following germination.

We sometimes find abundant natural reproduction of white pine under stands but usually we find this reproduction exhibiting poor condition and making little or no growth. We say that this condition is due to inadequate light. These experiments indicate that it is due to inadequate soil moisture at critical periods

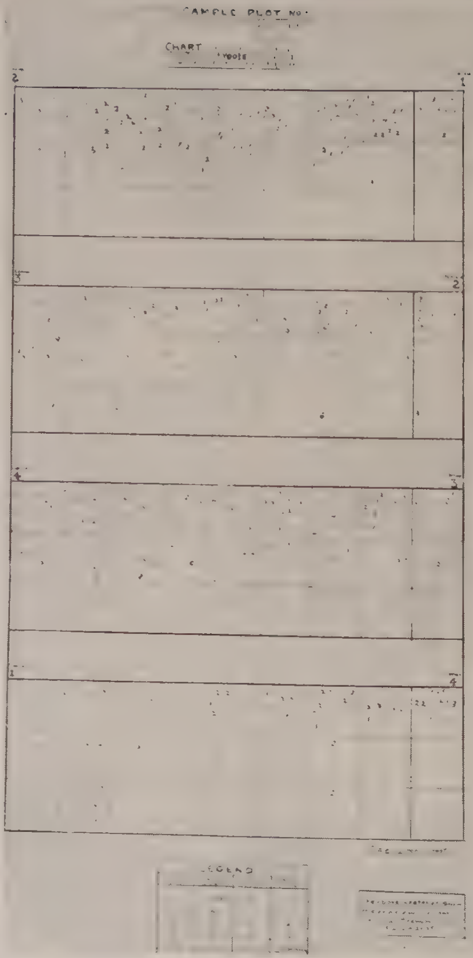
during the growing season and to mulch effect. We remove a part of the old stand and the reproduction surrounding the stumps of the trees removed shows greater vigor and accelerated growth not due, however, primarily, to increased light but to increased soil moisture.

Explanation of Plate I

Fig. 1. Sample Plot No. 5. Chart showing the number, size, and position of the roots severed on the 4 sides of the quadrat in trenching. August, 1922.

Fig. 2. Sample Plot No. 5. A chart of the vegetation showing the distribution and number of each of the 15 species present at the time of charting. August, 1922.

Fig. 3. Sample Plot No. 5. A chart of the vegetation showing the distribution and number of each of the 31 species present at the time of charting. August, 1925.



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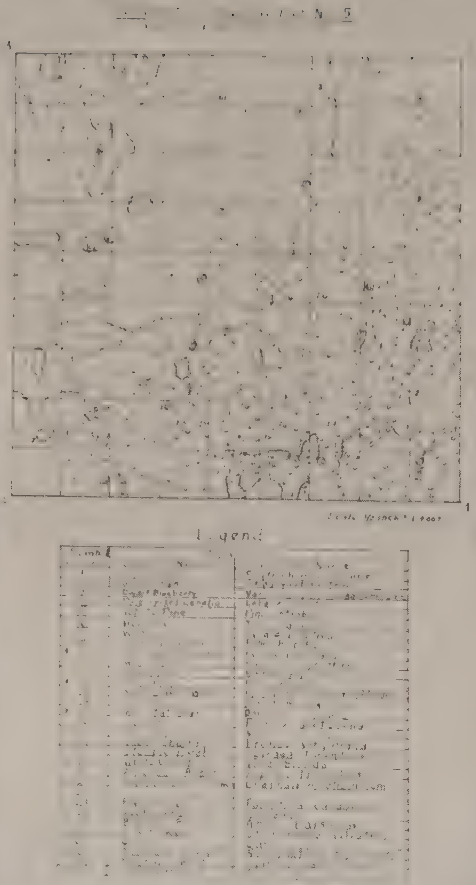

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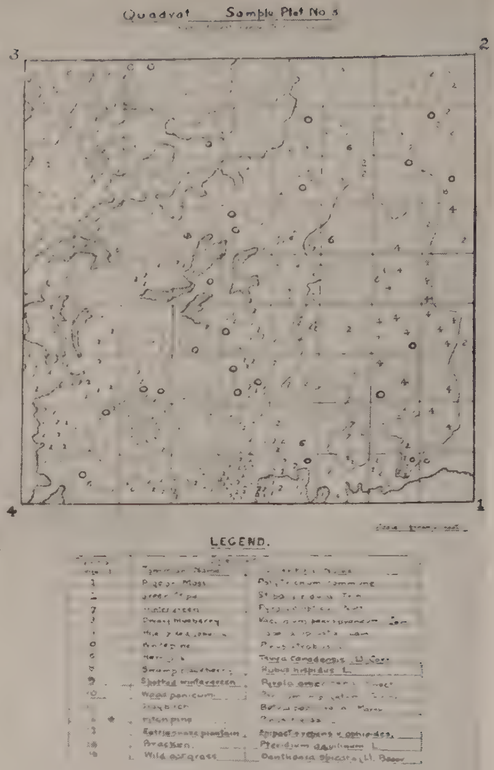

2

TOUMEY: VEGETATION OF FOREST FLOOR

CHANCE AS AN ELEMENT IN PLANT GEOGRAPHY[1]

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The leading principle of my researches on the plant life world of the Åland Archipelago has been my endeavor to give as exact an account as possible of the distribution, frequency, and abundance of the various species of plants and to make this account the basis of my exposition of the flora. With regard to the vegetation, I have sought a detailed knowledge of the composition and structure of the plant communities.[2]

It seemed probable that a thorough knowledge of the occurrence and frequency of the plant species in a given rather small phytogeographic district, such as Åland, would be of material assistance in the effort to define the laws regulating the general distribution of species. And further, a real insight into the causes of this general distribution is perhaps not possible without a thorough knowledge of the distribution in detail and its laws. It is, in fact, this detailed distribution of the single species in the single habitat that is the basis of the occurrence of plants in the large, just as the life conditions of human individuals and families are the foundations of the strength and development of human society.

The Åland landscape positively invites such a detailed study of the characteristics of the flora and the laws determining the distribution of plants,[3] for the reasons given in the next paragraph.

The islands of the Åland Archipelago form a rather sharply limited natural whole. Their total area is not too great for detailed investigation by a single botanist, so that the collection of exact and accurate material lies within the bound of possibility. On the other hand, the area is extensive enough to permit the laws regulating distribution, if there be such, to make themselves felt. Because the landscape is an archipelago it is possible to study the relations of occurrence of species within sharply defined areas, such as islands or headlands. A multitude of types of habitats and of plant communities are fairly represented in all parts of the archipelago. Consequently the Åland Islands offer great possibilities for the investigation of the natural laws of distribution and of the

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca New York, Aug. 18, 1926. (Read by A. G. Tansley.)

[2] I have already given an account of my motives for adopting this principle; see the references at the end of this paper as follows: 1915, pp. 40–41; 1917, pp. 614–615 = 1922, pp. 117–118(see also the preface to the German translation); 1921, pp. 27–28, 1925, pp. 9–15. For the development of my method of collecting the material see the following: 1917, pp. 484–486 = 1922, pp. 7–9.

[3] Compare my papers as follows: 1917, p. 480 = 1922, p. 4; 1925, pp. 15–16.

regular dispersal, and power of dispersal, of species in a region where no marked phytogeographical hindrances of any kind interfere with dispersal.

A detailed study of the occurrence and distribution of the plant species in the Åland Archipelago has brought to light a great many noteworthy relations, many of which would most certainly have been overlooked without such a thorough investigation. Not a few of these relations seem to find their natural explanation in some obvious circumstance.[4] Others, on the contrary, are quite inexplicable. And yet others impress us as a result of what I would call the merest chance.

Is it then possible to consider chance as an underlying cause of phytogeographical behavior? This question I shall investigate here in a preliminary way, but it cannot be done without first glancing at the phytogeographical factors.

It is very often difficult to throw satisfactory light on the effect of a so-called phytogeographic factor by the aid of examples and actual facts. To a certain degree such a task may be carried out experimentally, but only with regard to certain clearly defined factors. Very often one is compelled to locate and isolate the necessary facts among the multifarious phenomena of living nature. But always, if the effect of a particular factor is to be defined, the influence of other factors must be eliminated as carefully as possible, and this certainly is a very difficult task indeed.

One must not forget that the phenomena of the occurrence of species are never the results of a single factor, not even of the cooperation of a few effective factors, although some particular factor may appear decisive in a given case. Consequently, no single factor can be studied in isolation. It is thus very difficult to decide how far the influence of other factors may be safely eliminated without the risk of arriving at wrong conclusions regarding the phytogeographical significance of the factor under investigation.

We may therefore consider every phenomenon of distribution as the result of the cooperation of a complex of effective factors. These are due partly to the nature of the plant itself ("endogenous" factors,[5] that is, the constitution of the plant, or its individuality and possibilities of variation), and partly lie in the outer world ("exogenous" factors, that is, past or present climatic geographical, topographical, or biological conditions). Thus the factors involved are of very various character. The factors we have been used to regard as phytogeographical (exogenous factors) are also of very heterogeneous nature. Some are rather complex, others are probably more or less simple. Complete conformity of concepts can hardly be demanded so long as we have not been able to investigate, analyse, and classify the effective conditions satisfactorily. Collective concepts must exist side by side with simple ones, just as in floristic botany. In the complexes of effective factors, every particular factor acts in its own way. But only a definite cooperation of them all produces the necessary conditions for a given phenomenon of distribution.

[4] For reference compare my 1925 paper.

[5] See Engler, A. Pflanzengeographie. Die Kultur der Gegenwart 3rd Teil, 4th Abt. **4**: (cf. p. 222). 1914.

In its early beginnings plant geography had as its natural and fundamental aim the endeavor to ascertain the influence of the various phytogeographical factors, each taken separately. But plant geography cannot rest satisfied with this alone. The factors always work as a complex, a fact which inevitably demands an investigation, as detailed and as many-sided as possible, of the factors as they operate together in nature. This problem is naturally more difficult than the earlier one, and even its statement still remains uncertain.

The various phytogeographical factors may evidently cooperate in very various ways, in various combinations at various times, and first and foremost, in different relative strengths. Many factors quite naturally interact intimately and regularly (a great number always do so, for instance, temperature and humidity), while others influence each other under certain conditions only. In dealing with a given relation in which plants may occur, we are often able to point out definite factors that clearly enough have served as absolutely necessary conditions of the phenomenon in question, though it will probably never be possible to register them all with any certainty. When we preceive a particular mode of occurrence always reappearing under apparently similar circumstances, then we may feel justified in considering the phenomenon as following a law. On the other hand, noting that a species does not occur in a certain locality, we may often be able to explain this fact by proving that one or another absolutely necessary condition has been lacking, and the absence of the species is easily understood. In short, knowing the character of the species in question and the prevailing conditions of the habitat, we should be able to anticipate its absence. In other cases, on the contrary, all the various conditions for the establishment of a particular species appear to be present; we cannot find that they are fewer or less favorable than a similar locality where the same species actually grows. Consequently, the absence of the plant impresses us as inexplicable and accidental, that is, as being due to mere chance, when viewed against the backgroud of its occurrence in general. We may, perhaps, assume that of the various conditions for the establishment of this plant every one was, in fact, present, but the necessary combination or complex of them all at some important moment did not occur. Such a possibility must clearly always be likely, considering how heterogeneous are the conditions for the occurrence of a species. In this multitude of conditions, and of various possibilities for their combination into complexes, probably lies the chief cause of the accidental character that strikes us as being in so many respects characteristic of the conditions of occurrence—the stamp of mere chance. This leads us to the conclusion that we are justified in considering chance as an actual factor in phytogeographical distribution, and are even compelled so to consider it.

Since I thus have to allow "chance" a place among phytogeographically effective factors I must try to give an exact definition of my notion of the meaning of this concept.

The unreflecting view of the world characterizes as "accident" or "chance" only the exact antithesis of "according to law." "Whatever (it is supposed) cannot be ascribed to any law is attributed to chance" (compare J. S. Mill:

"A system of logic, ratiocinative and inductive"[6]). But science does not use the word in so negative a sense. Science always presumes that every phenomenon within its scope is a consequence of preceding causes. We may, however, use the notion "chance" in a relative sense. It is employed in this way (Rudolf Eisler, Wörterbuch der philosophischen Begriffe, 3rd ed., vol. 3, p. 1903, 1910) to indicate "das Eintreffen unbeabsichtigte, unvorhergesehener, aber kausal bestimmter Ereignisse, das Zusammentreffen zweier, in keinem (direkten) Kausalzusammenhang stehender Ereignisreihen, das einer Berechnung nicht zugänglich ist, so aber, dass sowohl jeder der Vorgänge, als auch das Zusammentreffen beider Kausalreihen im Weltzusammenhang begründet sein muss. Das Zufällige (s. Accidens, Kontingenz) in diesem Sinne ist das (für uns) nicht gesetzlich Bestimmbare, nicht zur Allgemeinheit und Notwendigkeit des Gesetzes Erhebbare. Eine grosse Rolle spielt der 'Zufall,' bedingt durch das Zusammentreffen von verschiedenen Kausalreihen sowie durch die Individualitäten, in der Geschichte (s. Soziologie)."

Especially in this last sense (Eisler, 2) the notion of chance has, in our practical theory of the world, a significance of great and profound importance, and thus we may apply it to phytogeographical problems. But then it will become necessary to recapitulate briefly a few circumstances already mentioned above.

When analyzing the notion of "cause" in its relation to the occurrence of a plant, we perceive that it is resolvable into a great number of individual circumstances or factors, of which every one exercises or has exercised a certain influence. But it is only as a complex that they constitute the necessary condition for the phenomena of the occurrence of the plant. Consequently it is not correct to assert a particular circumstance as cause. This would be an incomplete and therefore inaccurate statement. On the contrary, all the indispensable conditions must have been present during a certain, often rather limited, period of time.

The individual phytogeographical factors are in part of quite different nature one from another, and in such cases have no organic connection. They are obviously apt to influence one another in very different ways and with very varying comparative power. How this is going to happen in every single case is clearly quite impossible to anticipate. Thus no phenomenon is in every detail determinable by law. Likewise the result of the interactions of the factors cannot be something which in every detail can be "raised to the status of the generality of necessity of law" (Eisler). The result, in fact, bears the stamp of chance. The "chance" character here lies only in the manner or the time or the mutual interactions or combination of the effective factors, not in the working of the factors themselves.

In phytogeographical discussion the notion of "chance," consequently, means an effective complex of causes or, perhaps better, a combination of causes, so constituted that scientific research, for the present at least, is unable even to propound the problem of its ultimate essential dependence on natural laws. Chance thus appears as a unity and must be treated as such. We seem, at least for the time being, to be justified and even compelled to look upon it as an effec-

[6] The Silver Library Ed., 1906, Book 3, Ch. XVII, § 2, p. 345.

tive phytogeographical factor, along with the others. Up to now plant geography must acknowledge the impossibility of tracing every detail in the phenomena of occurrence back to some direct factor. It may be of some importance to phytogeography to establish this fact, and helpful too in clearing up our notions.

Our problem could consequently be thus stated: We might ask, what is the causation of a certain phytogeographical phenomenon, for instance—to take a simple case—the establishment of a particular species in a particular locality? The answer involves not only the special nature of the plant in question, but also its requirements with regard to climate, soil, geographical situation, topographical features, means of dispersal (for instance, the more or less favorably exposed situation of the habitat for the reception of seeds; winds and birds as carrying agents, etc.). All this has long been common knowledge. But it is theoretically possible, and experience seems to confirm it, that in another locality, where quite similar conditions clearly obtain, the plant in question has not established itself. In the first case we consequently must refer to one or more circumstances which have caused the directly effective factors to combine in such a way that the observed phenomena of occurrence are realized. Otherwise the explanation would seem to be incomplete. When it appears to be absolutely impossible to anticipate these phenomena of occurrence, then we may characterize the circumstance referred to as chance. When, on the other hand, we have been able to predict the occurrence, it must be ascribed to law.

But it will obviously be extremely difficult, perhaps impossible, by the aid of a mere definition to draw a line between what should be attributed to chance and what should be ascribed to law, when such complicated matters as the occurrence of living things are in question. The actual relations of chance and law may be so intermingled that we cannot disentangle them. For the task of our research it is, however, absolutely necessary to separate and keep apart what we presume to be defined by natural laws, and that which seemingly evades every possibility of positive explanation, at least at the present level of science. This last eventuality needs a label. As such we will use the term "chance." It would, no doubt, be of great importance to biogeography if we could estimate how wide and deep are the effects of chance. At the same time we shall in this way gain a deeper and broader understanding of the actual working of natural laws.

Let me add a few words of elucidation as to the notions of law and chance respectively.

As a result of my researches on the vegetation of the Åland Islands, covering a period of twenty years, I am now able to estimate with a high degree of accuracy (allowing an error of about 5 per cent) the number of species occurring in a certain area of normal forest meadow (Laubwiese).[7] This is doubtless a remarkable expression of the obvious fact that the development of the vegetation cover is regulated by natural laws. My knowledge of the structure of the vegetation-carpet (Pflanzendecke) further enables me to anticipate beforehand with almost absolute certainty (let us say as to 99 out of 100) a number of species

[7] See my treatise of 1915–1917 (=1922) and my exposition of this matter farther on.

(about one-tenth of the 300 odd species of the forest meadow vegetation) that will grow within the area in question. Likewise, I can with a certain determinable degree of probability (for instance, 90 per cent) predict the occurrence of other species, and with a somewhat lesser degree of probability the presence of still others, etc. I regard this, also, as evidently indicating a certain influence of natural laws. Nevertheless, it is to be observed that I can hardly predict with perfect certainty the occurrence of any single species as destined to appear in the area. And here again the effect of chance manifests itself.

It appears desirable, by a few concrete examples, to illustrate what has been said, and thus to demonstrate what seems to be the play of chance:

A rare species may be found on a certain spot, but is wanting in other quite similar places in the neighborhood. The plant has gained a footing in the district thanks to several favorable conditions. But the fact of its growing on precisely this particular habitat is, however, certainly due to some fortunate chance, because the possibilities of its occurrence here have manifestly been very few. Otherwise the number of habitats would have been much larger. It is, no doubt, also quite a matter of chance that just this habitat has been chosen, and not another similar one in the neighborhood. In this instance one feels justified in referring to chance as the ultimately effective phytogeographical factor.

Let us verify the aforesaid by casting a glance at the occurrence of rare species in Åland. Their stations (Fundplätze) are spread over the whole province, yet most abundantly in its western areas, where the number of species is largest. Otherwise, there is a total want of plan. For instance, out of the species belonging to the forest-meadow vegetation (Laubwiesenvegetation) *Alnus incana* and *Geranium columbinum* have established themselves in northern Åland in one single station each. The species mentioned below occupy only one or only a few habitats in the southern parts of the province: *Rubus pruinosus*, *Carex ornithopus*, *Stellaria Holostea*, *Corydalis intermedia*, *Sedum rupestre*, *Potentilla minor*, *Mercurialis perennis*, *Hypericum hirsutum*, *Campanula latifolia*. The following species are spread over different parts of Åland (the figures indicate the number of stations and the figures within brackets give the number of communes, where the habitats are established): *Rosa tomentosa* 4 (4), *Tilia cordata* 2 (2), *Phleum Boehmeri* 9 (6), *Brachypodium silvaticum* 8 (5), *Allium ursinum* 4 (3)., *Fritillaria Meleagris* 4 (3), *Cypripedium calceolus* 2 (2), *Ophrys muscifera* 7 (4), *Orchis mascula* 10 (3), *Herminium monorchis* 3 (1), *Cephalanthera longifolia* 9 (4), *Epipactis latifolia* 6 (4), *Vicia lathyroides* 4 (2), *Lathyrus silvestris* 6 (4), *L. niger* 7 (4), *Geranium dissectum* 8 (5), *Viola stagnina* 3 (2), *Ajuga pyramidalis* 3 (3), *Asperula odorata* 9 (5), *Crepis praemorsa* 8 (4).

In 1921[8] I called attention to the fact that the floristic composition of the southern forest-meadow vegetation becomes poorer in species the farther one advances towards the east on the Finnish mainland, a similar decrease in species having been observed towards the east of Åland. I am inclined to ascribe this phenomenon partly to the increasing distance from a center of dispersal in south-

[8] Die Entfernung als pflanzengeographischer Faktor. Acta Soc. pro F. et Fl. Fenn. 49, No. 1. 1921.

ern and central Sweden. Of the species of the Åland forest-meadow some go farther east than others. Many are established on isolated habitats situated more or less far towards the east. It has often been asked how these isolated habitats are to be explained. Why does just this particular western species occur on such a remote isolated spot, while the majority of more western species, commonly found in its company, are wanting? The phenomenon becomes clearer if we do not consider the species in question by themselves only, but rather as elements in a vegetation whose floristic composition has been greatly reduced in number of species during the eastward migration. The reduction has in a purely quantitative sense been determined by different conditions, among others by distance, while qualitatively the merest chance has obviously exercised a dominant influence. At times chance has favored or eliminated one species, at other times another. The element of chance is here very conspicuous.

Obviously chance has acted as a more or less effective factor in the production of the great majority, perhaps all, of the phenomena of distribution. Quite certainly it has played its part in the development of the vegetation on almost every spot where plants grow. As a rule, however, as is quite intelligible, it is not easy to estimate its effect, or rather the degree of its effect. To establish this I may call attention to a peculiarity of the distribution of species in Åland, which I have already (1917) described, and which is very convincing proof of the far-reaching role of chance. This peculiarity, however, cannot be elucidated in any other way than by a very detailed and thorough study of the occurrence of species.

Within one and the same phytogeographical district in Åland, the number of species is about the same in forest-meadow areas of equal size (without reference to whether they are identical or not on different areas) provided that only habitats of the same nature are compared (for example, islands, headlands, and the like). With increasing or diminishing area the number of species also increases or diminishes in a certain fixed progression.[9] The development of the species (broadly speaking) of a formation-type thus necessarily requires an area of a certain minimum size (Mindestfläche, minimale Fläche). For this smallest possible area I have, in the year 1917 (p. 618 = 1922, p. 121), suggested the term minimum area (minimi-areal—Mindestfläche), which has since been generally accepted in phytogeographical literature (see 1925, p. 38–40).

The various forest-meadow areas are consequently, according to their size, always inhabited only by a more or less considerable proportion of the number of species commonly occurring in the district, and therefore theoretically liable to invade the areas in question. Thus on every area a larger or smaller number of the species of the district are wanting, even many of the more widely distributed ones. This phenomenon is easily overlooked because of the difficulty, during casual observation, without making and comparing exact records, of retaining in memory the great number of species; but at times it will strike the eye by comparison between neighboring areas of the same nature. In addition to the occurrence of a predominant contingent of species common to all the

[9] 1915–1917, p. 509, 617 = 1922, p. 28, 120. See further, 1925, p. 31, 32–43.

areas, there is always a number of remarkable differences, which could hardly be expected.[10] The causation of these differences obviously lies, as a rule, in the chances and opportunities of the process of occupation. Only a certain number of the species available for colonization will be able, that is, will find time to, colonize the areas in question. Others arriving later, meet a closed vegetation. It depends in a high degree on chance whether a plant will succeed in gaining a foothold in time, before the vegetation becomes closed and a more or less effective obstacle to the entering of new elements is established. Evidently species with small power of dispersal are those which are most likely to be excluded by this play of chance, but at times the same fate befalls even the most abundant species.[11]

It has already been mentioned that the floristic composition of the vegetation in various similar places (islands, headlands, and the like) present differences, obviously due in a high degree to chance. A comparative investigation of the frequency relations of the species of these particular areas will disclose the fact that a given plant will occur with a very varying degree of frequency on the different areas, even if the conditions of the habitats are all equally suitable. Likewise, even if the natural conditions are equally suitable, the particular species will be found very unevenly dispersed over various parts of one and the same area.[12] This, too, of course, is due to the play of chance. It happens not infrequently that some particular species occurs with a quite unexpectedly high frequency and abundance within a given area. This is surely due to some especially favorable incident. On another area a similar observation may, perhaps, be made regarding some other species.

The want of uniformity in floristic composition that exists between the various forest-meadow areas (as well as between their various parts) finds an analogy in the particular habitats of the same type. The vegetation covers of these habitats show great differences in their floristic composition, notwithstanding the similarity of the conditions. This holds good, even if the habitats lie close to each other. The cause of this is, of course, the same as that which is responsible for the differences of the various areas.[13] The particular habitats are seldom large enough to offer sufficient space for all the species belonging to the type of habitat, that is, the particular habitat does not occupy the necessary "minimum area."[14] Thus the species available for colonization of the habitats

[10] I refer the interested reader to my studies of the forest-meadows, III, Ch. VII (Comparative analysis of the flora of the various special areas), where a comparison in this sense between the number of areas is rendered on p. 577–580 = 1922, p. 86–89. (See further 1917, p. 479 = 1922, p. 3).

[11] See 1925, p. 41; 1915–1917, p. 618–620 (= 1922, p. 121–123). See the exposition of the rare species in the author's treatise of 1925, p. 117–123; chapter X in his work of 1917 (= 1922); further, his points of view with regard to the flora of Gotland (1917, p. 630–632 = 1922, p. 131–134); see also 1915-1917, p. 580–585 (= 1922, p. 89–93.) where are cited a number of species very common in Åland but which without any visible cause whatever are totally lacking on one or other of the special areas described in the work mentioned. Finally, I refer to my treatise of 1925, where I describe some peculiarities in the distribution of a number of species, seemingly due to mere chance.

[12] 1917, p. 594 = 1922, p. 100.

[13] 1915, p. 38–40; 1917, p. 594–597 (= 1922, p. 100–102); 1925, p. 41, 133–134.

[14] 1917, p. 618 = 1922, p. 121.

must unavoidably come into competition with one another, and those with the greatest power of dispersal have, of course, the greatest chance of victory. As soon as the carpet of vegetation has become closed, the chances of obtaining a foothold are very small indeed.[15] Thus many species will be excluded only because they have "arrived too late."[16] In the competition here indicated chance will play a very important role, the more so the larger the number of competitors and the smaller the available area. Where the individual homogeneous habitats are small, but the number of species is considerable, as in the case in Åland, there the carpet of vegetation bears a strongly marked stamp of pure chance. In such cases the carpet of vegetation of the so-called habitat "does no more than illustrate how, under certain given conditions (partly on the spot in question, partly in the surroundings), the floristic composition of the carpet of vegetation may possibly develop" (1917, p. 620 [=1922, p. 123]; also 1915, p. 39). "On an absolutely similar place a few steps further on the vegetation may in one or more respects have developed in quite another way; exact equivalents are, on the whole, not to be found" (1917, p. 596 [=1922, p. 102]).

My presentation of these matters has, I hope, made clear how very important is the influence of chance on the development of the various plant communities, the colonization of the habitats, and the spread of the vegetation and the flora over wider areas. It is, of course, very hard to ascertain in every instance how great this influence of chance really is. The structure of the carpet of vegetation is a result of such a host of varying circumstances. It is very difficult to isolate, survey, and measure the separate effect of every one of them. The distance from the parent plants, the exposure to colonization, the size and character of the area under consideration, the time allowed for the development of the vegetation[17]—each one of these factors exercises its special influence. The final and decisive factor, however, is exactly how many and which the available species have been (see 1917, p. 592, 619-620 [=1922, p. 98, 122]). Their relative power of dispersal under the prevailing geographical and topographical conditions is the characteristic most likely to exercise the deciding influence on the floristic composition of the areas to be colonized (see, for instance, 1917, p. 592 [=1922, p. 98]).

It is manifest that this play of chance, notwithstanding its powerful influence on the quality of the vegetation cover, leaves the number of species largely unaffected. Here we obviously have the reason why, for instance, the available species within the same plant communities are, as to the number of species,

[15] 1917, p. 593 (=1922, p. 98–99).

[16] 1917, p. 619 (=1922, p. 121); 1925, p. 38.

[17] This circumstance is often overlooked. The development of a certain plant community requires, of course, under certain conditions a certain time. To indicate the shortest possible time during which this development can take place I would suggest the term minimum time, in analogy with my earlier term minimum area. It is naturally of the greatest importance to know this minimum time.

Åland offers a very suitable field for the study of time as a factor on account of the secular rising of the land for thousands of years past and the innumerable islets, islands, and skerries, whose age can be estimated almost exactly (see my treatise of 1925, dealing with this secular land-rise).

distributed in so remarkably constant a way over the various degrees of frequency (compare 1917, p. 622–623 [=1922, p. 1925]), although the species themselves may vary considerably. Here, too, lies the cause of the fact that numbers of species common to 2 or more communities coincide so strikingly in various comparable areas, even if the actual species common to the several communities vary from one instance to another.

The significance of the number of species, as distinct from their nature, as a phytogeographical character increases when seen against the background of the foregoing considerations.

What position has plant geography taken up with regard to chance as an effective factor? In the introduction to his Handbuch der Pflanzengeographie, 1890, Drude defines the aims of plant geography in the following words:

"Unter Pflanzengeographie verstehen wir die wissenschaftliche Betrachtungsweise der Flora im Lichte der physikalischen Geographie: ihre Aufgabe besteht in der Erforschung der Gesetzmässigkeit der verschiedenartigen Verbreitung von den Elementen dieser Flora über die Erdoberfläche, und in der Erforschung der Wechselbeziehungen zwischen der Erscheinungsweise des Pflanzenlebens und seinen mit der geographischen Lage sich verändernden aüsseren Bedingungen."

Phytogeography tries to ascertain the laws of the distribution of plants. The more scientific research develops, the more of those phenomena which formerly seemed enigmatical have found their natural explanation. In many cases science has established evidences of regulation by law, where earlier confusion appeared to reign. Thus we have been induced to expect a positive explanation of almost every phenomenon of distribution. In this we have, however, obviously gone too far. Chance is, in many cases certainly, revealed as the final underlying cause of the phenomena. Indications of this idea are not lacking in literature. But plant geography has doubtless been guilty of an omission by not taking into account chance as a real working factor, with wide scope for its influence, demanding investigation as much as any other existing factor. The chance factor has thus not been taken up as an independent phytogeographical problem. Consequently, for the solving of this problem there has been no collection and sifting of material. This becomes evident by a glance at the summaries in the current handbooks of plant geography. One looks in vain for "chance" among the factors discussed. That chance must in fact be the basis of multidinous conditions of occurrence is, however, quite natural. It is quite as natural as the fact of chance having played an important role in the development of the history of man, which makes it a significant factor to be reckoned with in the future development of human history.[18]

[18] The part chance plays in moulding the history of man is still greater than its influence upon the development of vegetation, because the human reason comes in here as an extremely important factor, causing human individuals to react differently in the face of similar situations.

It may perhaps be said that in making chance a geographical factor I introduce a concept which can easily be made a "rubbish heap" on which any phenomenon of distribution that is difficult to explain may easily be thrown. I can only reply that reference to chance as a ground of explanation naturally demands as careful an investigation as reference to any other factor.

That chance as a phytogeographically effective factor has, on the whole, escaped notice, has its immediate cause in two circumstances. Partly, it will always be hard quantitatively to determine the scope of its influence, which, besides, in many cases will be difficult to isolate at all. Partly, the unravelling of chance as an effective factor of large and general bearing requires considerable and detailed material—as does, for instance, distance, taken as a factor. The presentation of such material has, so far, not been included in the aims of plant geography (compare my exposition 1921, p. 51).

In this respect as well as in others a small but very thoroughly investigated area clearly offers greater opportunities than does a large one for the propounding and development of this problem. By surveying and measuring the phenomena of distribution and their causes in a larger district one hardly obtains equal possibilities for estimating and comparing the factors at work in different localities. And, consequently, it is harder to eliminate certain factors as being of equal value, or indifferent, so that the special effect of others may appear more plainly. An instance will illustrate this. Two different areas show certain differences in the floristic composition of their vegetation. On one are to be found one or several species which are lacking on the other. The causation of this is to be investigated. If these areas border on each other, it will be easier to elucidate the effective factors than if they were situated at a distance. Many of these factors (for instance, the climatic ones and those connected with the geographical situation) can be eliminated as of manifestly equal value, and the problem thus be limited to a smaller number. In the Archipelago of Åland, which is of limited area and composed of hundreds of large and small islands, islets, and skerries, varying from a few square meters to several square kilometers, often lying very close to each other, there are great opportunities of finding in the immediate neighborhood of each other regions of geographically and topographically the same nature and possessing a vegetation so much alike that the differences will be detected only after a very careful and detailed investigation. Also, there are islands seemingly alike in all other respects, but differing regarding one or another important circumstance. In other words, nature itself generously provides natural test-areas (Probeflächen) for the study of several problems and questions. Vegetation and flora here both strongly indicate chance as an effective factor.

At nearly every step in the landscape of Åland we find features of the composition of vegetation and flora, which, seen against the background of conditions in other habitats close by, impress us as due to chance.[19]

It is obvious that in the study of the general features of the vegetation of a large area, we incur a very great risk of disregarding chance in the details, because these may counterbalance one another, thus making a uniform total impression.

This paper does not pretend to be anything more than a preliminary communication and an encouragement to the study of a problem which has hitherto

[19] 1925. p. 31, 41, 61, 91, 112, 120–121.

been overlooked, but which is quite certainly of remarkable significance. Its scientific treatment will certainly prove anything but easy.

I have not found it possible nor even necessary in this paper to expound the problem of "chance" as a biogeographically effective factor against its (so to speak) philosophical background, nor to analyze or develop the concept of "chance." This is rather a task for the philosophers. In the present state of the problem I am satisfied to adopt Eisler's formula, quoted above. The complete formulation of the problem demands also more facts and more extended study of the matter than is at present available. I think, however, that the preliminary form I have here given the subject may prove sufficient as a foundation for discussion.

My view is, that "chance" must be given a place amongst the biogeographically effective factors and as such be an object of attention and research.

A critic may perhaps be inclined to object that we have no guarantee that what impresses us as mere chance, may not, as science develops, prove resolvable into various factors or reveal itself as capable, in the particular instance, of being brought into line with recognized natural laws.

Well, be it so! Scientific discussion has always progressed from a superficial and rather loose grip on its subject to a deeper and closer understanding. But we shall never arrive at a thorough analysis or an eventual solution of the problem of chance if we do not undertake the study of it in the only way that seems at present available. Consequently, biogeography has to tackle the problem by isolating and measuring the phenomena which, in the best analysis we can give, appear to be determined by chance. In doing this we shall at the same time be forced to estimate very critically the effects of the other positive factors. In this way we may obtain an idea of the comparative importance and scope of the play of chance.

List of Previous Publications of the Author Bearing on the Subject of the Present Paper

Palmgren, A. ('15–'17) Studier öfver löfängsområdena på Åland. Ett bidrag till kännedomen om vegetationen och floran på torr och på frisk kalkhaltig grund. I. Vegetionen (1915), II. Floran (1915), III. Statistisk undersökning af floran (1917). Acta Soc. pro Fauna et Flora Fennica, **42**:—634. 1915–1917.

—— ('22). Über Artenzahl und Areal sowie über die Konstitution der Vegetation. Eine vegetationsstatistische Untersuchung (Übersetzung von des Verfassers: Studier öfver löfängsområdena på Åland. III. Statistisk undersökning af floran, 1917). Acta Forestalia Fennica **22**: 1–136. 1922.

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THE PRESENT STATE OF GEOBOTANICAL RESEARCH IN SWITZERLAND[1]

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I. Introduction: Climate and Corresponding Vegetation and Cultures

Before I can deal with geobotanical research proper I must begin with a brief sketch of the characteristic factors of the Swiss habitats and their general effects on the Swiss vegetation and on farming.

The very mountainous configuration of Switzerland gives rise to a great variety of climates and of soils on a small area. As early as the year 1768 our grand old plant geographer Albrecht von Haller ('42), one of the founders of plant geography, gave a good account[2] of the climatic belts (altitudinal zones) of Switzerland, comparing them with the great regions of Europe determined by latitude. He says that Helvetia represents nearly all regions of Europe from arctic Lappland and Spitzbergen to warm temperate Spain; in America one might say from Labrador to Florida, or from Alaska to California. We have climates ranging from the warm temperate to the most frigid, and from quite oceanic to markedly continental.

Our excellent new precipitation map by Brockmann-Jerosch ('25) (wall map in 1:200,000, published in his book in 1:600,000) may serve as a basis for explanation. Climatically the largest part of Switzerland depends on the westerly rain-bearing winds. In the first instance they strike the Jura mountains in the west. Much rain falls on them and on the French country to the west of them, because the rain-laden atmosphere is thus backed up by them. The eastern slopes of the Jura and the flat land beyond are dry, since they lie in the "rain shadow." At a higher level the rain-bearing winds pass eastward over the Jura and strike the much loftier Alps giving much water to their western slopes and leaving very little for the central valleys.

The southwestern part of the Jura is rather high, and while its northwestern slopes are very cold and wet, the southeastern are very warm and dry, a country of excellent wine; eastward the "Prealps" receive much rain only in the upper altitudes; the valleys are dry. But the northeastern Jura is much less lofty, so that the contrast of wet and dry sides is less pronounced and the good vineyards

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 18, 1926.

[2] Even in Haller's first edition, of 1742, there are indicated many of the lines of division of vegetation.

have disappeared. The rain-bearing winds blow at a lower level, so that the northeastern "Prealps" get rain both on the hills and in the valleys, and the valley system does not show so distinctly on the rain map. Still farther to the north we have again a drier country owing to the protection of the Black forest.

The southern slopes of the Alps show quite a different climate. Here the southerly rain-bearing winds from the Mediterranean bring heavy precipitation, which falls rapidly, leaving the country exposed to the strong southern sunshine for long periods. In spite of very high precipitation (an annual mean of 1910 mm. or about 77 inches) the region of Locarno, of recent political fame, is a very sunny country. This specialized climate, different from all others, is called the Insubrian. I refer you to the big, new agricultural and vegetational map of Switzerland by Brockman-Jerosch, which will appear in the second installment of his book on Swiss vegetation ('25), the chapter on biotic factors, or culture practices of the country.

In Roman times forests must have covered great parts of the country. With increasing population much of the forest was felled, especially in the thirteenth and fourteenth centuries, and farming increased more and more, cultivation by sowing (Saatkultur) and by hoeing (truck farming, potatoes, vegetables, etc.), as well as grassland culture. At the beginning of the nineteenth century Switzerland produced nearly enough grain for her own use. International trade has led to the importation of cheaper grain, so that now we do not produce more than enough for about 1½ months of the year. In the drier regions, of course, grain production has maintained itself best. These are the areas with improved practice of triennial rotation of crops on permanent arable land (verbesserte Dreifelderwirtschaft, régime triennial amélioré). In the northeastern and higher parts the rotation runs: first year, winter wheat (*Triticum aestivum*); second year, oats (*Avena sativa*) or summer barley (*Hordeum distichum*); third year (the former fallow year), potatoes or red clover (*Trifolium pratense*). This may be called the summer grain sequence. On lower-lying land to the west we find the winter grain or rye sequence with first year, winter wheat (also *Triticum aestivum*); second year, winter rye (*Secale cereale*); and third year, potatoes or red clover.

The greater part of the midlands from Lausanne to Aarau has clover grass culture, mostly derived from the former practice of triennial rotation of crops. The same ground bears alternately arable field and fodder culture. The country of the foothills of tertiary Molasse and the limestone "Prealps" is mostly under permanent meadow, which has encroached more and more on other kinds of cultivation. On the map you may see especially well the encroachment of meadow on the area of the triennial rotation of crops in the cantons of Zürich and Thurgau. All these meadows, together with the subalpine and alpine pasture, provide fodder for cattle raising, a famous Swiss industry. But we have too little fodder; concentrated fodder (Kraftfutter) must be imported, and with 1,500,000 to 2,000,000 head of cattle we have far too little for our dense population.

Switzerland may be called overpopulated, since there are 94 inhabitants to the square km. compared with only 12 in the United States—which must be considered a very sparsely populated country, with only one-eighth of the Swiss population per unit area. In other words, the United States should have 880,-000,000 inhabitants to be as well populated as Switzerland. I do not wish you all the 880 millions but you may increase by a few hundred millions and not be afraid of overpopulation by immigration. Even so, Switzerland has about 25 per cent of unproductive land—rock, glacier, and water surface. In one of the more industrial states with little unproductive land, the state (Canton) of Zürich, the population reaches a density of 312 to the square km. If we compare this with the industrial state of New York, in which we have the pleasure of being just now, there are 81 inhabitants to the square km., which means a density in the state of Zürich about 4 times that of the state of New York.

Our country has about 25 per cent of unproductive land, only 12 per cent of field crops (of which truck farming is a larger percentage than cereal production), 0.5 per cent of vine culture (which, small though it may appear, means an important industry), and 40 per cent of grasslands (more than half of the whole productive area). The remaining 23 per cent is forest.

If I try to form a parallel between these types of cultivation and the natural vegetation we obtain the following scheme:

<table>
<tr><td>Viticulture corresponds to prairie, Hartgraswiesen, Garide</td><td rowspan="4">Originally mostly forests, some reclaimed swamp and moorland</td></tr>
<tr><td>Arable field culture corresponds to montane dry meadows, Trockenwiesen (Brometalia)</td></tr>
<tr><td>Fodder culture corresponds to manured meadows, Fettwiesen (Arrhenaterion)</td></tr>
<tr><td>Litter culture corresponds to wet meadows, Humidiprata (Molinion)</td></tr>
<tr><td>Pasture corresponds to alpine grasslands, mostly dry</td><td></td></tr>
</table>

The rapidly varying vegetation on small areas positively forced upon us the development of ecological and phytosociological research.

II. Historical Sketch of Swiss Phytosociology

Before proceeding to consider the present state of Swiss phytosociology I will just mention briefly the beginnings of phytogeographical work in Switzerland. Conrad Gessner, 1516–1565, searched the Alps for plants and made reports of botanical excursions on the Pilatus, the Stockhorn, Niesen, etc. The great chronicler Josias Simler, in 1574, in his "Commentarius de Alpibus," wrote about endemism and about the distribution of different trees, of pasture, etc. Johann Jakob Scheuchzer, 1671–1733, made long journeys through the Swiss Alps and described the vegetation. The great botanist, physiologist, and poet, Albrecht von Haller (1708–1777), introduces real phytogeographical ideas. He traced the belts of vegetation through Switzerland and gives good descriptions of them, which on the whole are still valid ('42, '68). In 1831 Hegetschweiler,

after having finished a flora, wrote out his manifold observations in a book which we may call a real ecology of vegetation. A few years later, in 1835, appeared the beautiful monograph of the Sernftal by Oswald Heer ('35), quite in the form of our modern monographs on areas, in which the abundance and sociability of every plant in every habitat are given, and real communities are described, for example, the famous "Schneetälchen" or "snow flush vegetation." This work had no successors at the time, and it was not till 60 years later that Schröter brought this kind of research into the foreground in his "St. Antöniertal."

With great strides I pass to the living scientists and the present day. In 1879 Hermann Christ, our revered veteran still living in Basel, gives us a modern description of vegetation of Switzerland in his "Pflanzenleben der Schweiz." Stebler and Schröter ('92) studied the meadows in a broad sense. Schröter's meadow types are a very thorough morphological and ecological study of these communities, a fundamental work which will always have to be consulted. These "types" are considered as indicators for agriculture, and natural successions are studied to give the farmers hints for good artificial successions. The authors describe 21 types, say associations, with 68 "Nebentypen," say subassociations or consociations. Most of them remain valid to the present day.

In the "Vegetation des Bodensees" Schröter ('02) gives a systematization of the new science of plant communities and calls it Synecology. Since then this science has widened, and we now call the science of communities "Sociology," of which chorological, ecological, genetical sociology are chapters; in the English language the ecological chapter still gives the name Ecology to the whole subject, *pars pro toto.*

Before instituting this taxonomy of synecology Schröter had worked out communities empirically. In 1895 he published his monograph on the "St. Antöniertal im Prättigau" in its cultural and phytogeographical relations. This is the first of a long series of similar monographs by his pupils, and later on by pupils of his pupils, and by others. The monographs are distributed over many different parts of Switzerland, as the accompanying map shows. From this point knowledge of the vegetation of Switzerland, especially of the Alps, grows at an increasing pace. Methods of investigation improve, problems are grasped more and more intimately. The works are, of course, of very different value: one brings new ideas, another works on principles already established; one lays more stress on pure science, another on silvicultural and agricultural data. Several departments of geobotany are usually dealt with in these monographs; four topics especially characterize the early ones: there is first a floristic part with a detailed catalogue of species of the area studied; secondly, a synecological (sociological) part; thirdly, a genetical section dealing with the immigration of the species; and lastly, a cultural or economic section, agri-, silvi- and especially alpi-cultural (Alpwirtschaft). The first chapter deals with habitat—climate, soil, etc. The cultural chapter shows how geobotany on purely scientific grounds links with practice; in fact, silviculture and agriculture are the applied ecology of forests and meadows.

The first of these papers appeared in 1900, Waldvogel's "Lautikerried und er Lützelsee"; in the following year the "Vallée de Joux" by Aubert ('01), the lvibotanical monograph of the Bergell by Geiger ('01), and the Curfirstengebiet y Baumgartner; then a little later the Sihlseegebiet by Düggeli ('03), the ottoceneri by Bettelini ('05), the Bergünerstöcke by Grisch ('07), and the uschlav by Brockmann-Jerosch ('07). Thus were laid the foundations of the -called Schröter school or Zürich school. With Brunies ('06), Geilinger ('08), nd Jäggli ('08), similar research begins in the sister institution, the University Zürich (Prof. Schinz). Floristic predecessors we find in Briquet's Mont Vuache 94), in Hegi's Tösstal, etc. Of course, I cannot cite all. The field has broadened nce then. Special questions have been picked out; for example, the ecology

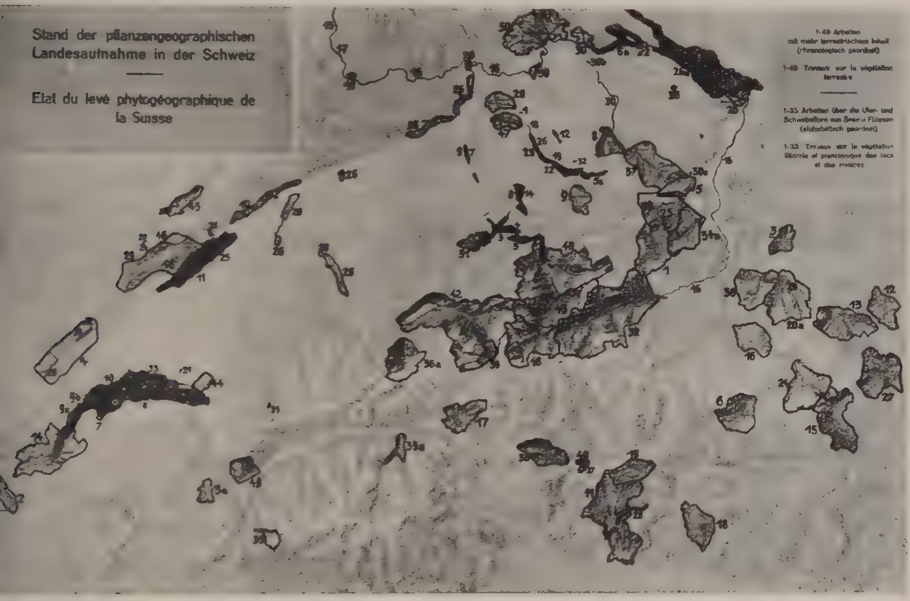

Fig. 1. Phytogeographical areas studied.

rubble plants; in the Albula region, by Eugen Hess ('10); in Les Plans, by uarles van Ufford ('09); the ecology of rock plants, on siliceous soils, by Wetter 18); and on calcareous soils, by Oettli ('03).

As interesting for Americans, it may be cited that succession, while always nsidered, was put into the forefront of researches by Siegrist ('13), in the areauen region, by Furrer ('14) in the Bormio region, and by Lüdi ('18, '20) in e Lauterbrunnental.

The cryptogamic communities, not of primary importance in Swiss vegeta-on, had to wait until the more important phanerogamic communities were rveyed, but now the former also are being intensively studied, especially by d. Frey ('23) in the Grimsel area, and by workers whose papers are now being epared for publication. Since the cryptogamic communities are of great im-ortance in Scandinavia, their study has taken a foremost place in that country.

III. Methods of Research

With continued work the methods of research continually improved and eventually plant communities were given morphological and taxonomic treatment of their own. In this work Switzerland has taken a leading part. The same is true of America and other countries also, but I shall be forgiven if I confine myself to the proper subject of my communication and speak only of the Swiss papers. Many of the monographs, especially the larger ones, contain chapters on these topics, and in later years papers dealing with them have become numerous.

On the foundation of Schröter's ('96, '02) classification of communities research methods are treated in Brockmann's ('07) Puschlav and Rübel's ('11) Berninagebiet and a system was worked out by these two authors ('12) jointly in their classification of plant communities from the standpoint of physiognomic ecology. I gave an application of it (Rübel '13) in the handbook of natural sciences.

Our young Swedish colleagues reiterate that their modern school of plant sociology is based especially on these two monographs (Bernina and Puschlav, Brockmann '07, Rübel '12). Many have contributed since; nearly every paper brought supplements. I name only the questions of principle in vegetational research by Gams ('18), the principles of taxonomy of plant communities on a floristic basis by Braun-Blanquet ('21), Braun and Pavillard's ('22) vocabulary of plant sociology. Furrer ('22), Lüdi ('20), Braun and Furrer ('13), and Frey ('22) have written systematic essays on questions of succession. The history of the growth of geobotany, especially of sociology, I have given ('17, '20, '20a) in my papers on beginnings and aims, on the development of sociology and on the development of the morphology of communities, showing how various countries have contributed to the subject. In my book ('22) on geobotanical research methods I tried to bring together the best and the most durable of the multitude of methods proposed. A concise selection I gave ('25) in the propositions for the survey of beech forests which I wrote at the request of the I.P.E.

What we want to know nowadays about an association, its morphology, ecology, and genetics (succession) I will concentrate in a few questions. In the *Morphology* we study the whole organization of the plant community, the present components (species), their distribution, diffusion, quantity, regularity, life form, and rhythm. We may ask the following statical research questions:

1. What species occur in the association? List of species.
2. What importance should be assigned to each species by its form or number of individuals? Quantitative proportions.
   a. How many individuals of the species occur? Abundance.
   b. To what extent does the species preponderate? Dominance and degree of cover. These two questions must not necessarily be separated, they can be answered with one scale from high abundance to occasional and rare.
   c. In what sort of grouping does the species occur? Sociability. It occurs as a big herd, in crowds, in troups, in little groups, or isolated.
   d. How does it thrive? Vitality. A species may thrive characteristically in one association, but still occur in another in a reduced or sterile condition; so it is desirable to know this vitality.

How regularly is a species present in the different notations (Aufnahmen) of the same community? Constancy. This is one of the most important synthetic characters and can be expressed in a scale. Constant species have a high sociological value.

How strongly is a species bound to certain communities? Exclusiveness or fidelity. If a species occurs only in one single association, it is exclusive (5) and therefore of high sociological value as a characteristic. A somewhat less exclusive species may be called elective (4); then "preferring" (3); others grow indifferently in many associations (2); others occur casually and may be regarded as alien to the association (1). "Differential" species mark the difference between otherwise narrowly related communities. Fidelity may occur in regard to one association or in regard to a group of associations (verbandstreu), or in regard to a higher unity only; say exclusively in forests in general, exclusively in deciduous forest, exclusively in beech forest, exclusively in a Fagetum alliosum.

What live forms occur in a community and what significance have they? Life form. Raunkiaer's life forms amended by others are commonly used.

Into what ecological groups may the community be subdivided? Synusia.

Does the community consist of different layers? Layers. Not all plants of an association exist under the same ecological conditions, especially in different layers various factors act differently. In a Phragmitetum we have a reed synusia, a limnaeid synusia, in the water hydrocharid synusia, in the mud the Schizophyceae synusia.

What changes occur in the life of the species during the year? Periodicity, rhythm.

What phenological groups does the community possess? Aspect.

After the morphology the *successional* questions must be considered. We ıust find out if we have to do with initial, medial, or final communities. Every ssociation has an initial phase, an optimal phase, and a terminal phase. The ssociations combine to form series, naturally progressive to the climax, but also egressive ones.

Every species of an association has a certain constructive value (Bauwert, übel '25) or dynamic behavior (comportement dynamogénétique, Braun and avillard '25). If it helps to make the association optimal, then it is constructive, r it is conserving or consolidating or simply neutral; or if it helps to change the ssociation, it is destructive.

I need not emphasize the *ecological* factors, the methods here are generally ıternational; we all study heat, light, etc. Every one knows the famous atıometers of Livingston. Everybody studies soil factors; in recent years much f hydrogen ion concentration, and so on.

Not so thoroughly studied often are the *biotic* factors; the strength of cometition of each species, competing for room of roots, of leaves, etc.; the useful r hurtful effect of parasites or saprophytes, or of animals. Here we must also tudy the effect of man and his domestic animals, his activities, such as grazing, ıowing, watering, and other agricultural and silvicultural factors.

For the study of forests (see especially Rübel '25) we must know the *silviultural practice*, these are questions of applied ecology:

How did the forest originate?

Was it (1) from artificial sowing, (2) by plantation, (3) from reproduction by coppice shoots, or (4) from natural rejuvenation by self-sowing, and in the latter case with or without some thinning out (Lichtungshieb)?

We must know whether the forest is managed as high forest (Hochwald) or as coppice (Niderwald) or copse that is coppice with standards (Mittelwald). If a forest is cut clear (an ancient form of

forestry practice) all ecological factors change, but with the modern form of "plentering," that is, taking out only the old trees, so that all tree ages are always present, there is little disturbance of general ecological conditions.

3. We must measure the diameters and ages of the trees.
4. Density should be observed, that is, to note if the forest is "densely closed," or with varying degrees of openness.

It is only recently that these precise questions have been clearly put and most of the older papers do not consider them all, so we do not yet know the Swiss associations so well as would be desirable. There is much still to do in this direction, as there is indeed everywhere and in every science.

Vegetation maps are well advanced in Switzerland. I cannot go into details about the cartography now. I must refer to the papers about it (Rübel '25, etc.) and especially to the maps themselves. Schröter gave a full account of vegetational mapping at the Brussels congress of 1910 with a large exhibit. I have hung up here some of our most recent maps.

After these few words about the methods adopted I will give a very short synopsis of the vegetation we work on. Though Switzerland is a small country, the climate and soil vary through a wide range from the warm southern lakes, only about 200 m above sea level, to the glaciers and ice peaks up to 4600 m.

IV. Plant Communities of Switzerland
Synoptical List and Sketch

Here I shall try to give a list of communities from old and new surveys, recognized both by the more perfected and by the older methods, their modern names given partly by their authors, more often adapted by myself. Not all the communities mentioned will keep their rank when worked over again by our modern methods, some will be degraded in rank, others will disappear as unnatural or accidental. I will not read the list, but distribute it and give a short account of the most important communities.

Aestisilvae. *Fagion silvaticae medioeuropaeum.* Fagetum anemonosum nemorosae, Fagetum alliosum ursini, Fagetum acerosum pseudoplatani, Fagetum abietosum, Fagetum taxosum (?), Aceretum pseudoplatani. *Quercion.* Quercetum pratosum, Quercetum sessiliflorae (geogr. var. with Ostrya), *Castaneion.* (Castanea high forest, "Selva"), (Castanea coppice, "Palina"). Carpinetum betuli, Acereto-Fraxinetum, Cariceto remotae-Fraxinetum, Birch forest, Alnetum incanae (Auenwald), Cariceto elongatae-Alnetum glutinosae (Bruchwald "Carr").

Aciculisilvae. *Pinion silvestris.* Pinetum silvestris fruticulosum, Pinetum silv. engadinensis, Pinetum silv. with "xerotherm" relicts, Pinetum silv. festucosum capillatae. *Piceion excelsae.* Piceetum exc. myrtillosum, Piceetum exc. abietinosum. Abietetum albae, Laricion europaeae. Laricetum eur. pratosum, Pinetum cembrae fruticulosum. *Pinetum montanae.*

Aestifruticeta and Aciculifruticeta. Coryletum avellanae, Rosetum, "Muschnas," Alnetum viridis, Saliceta (several), Salicetum waldsteinianae, Salicetum helveticae, Hippophaetum rhamnoidis, Pinetum montanae prostratae (Legföhrengebüsch).

Lauri- and Ericifruticeta. Buxetum, Garide. *Dwarf shrub (Zwerggesträuch).* Rhododendretum ferruginei, Rhododendretum hirsuti, Vaccinietum myrtilli, Vaccinietum myrtilli, subass. vitis idaeae, Callunetum subalpinum, Callunetum subalpinum subass. Ericetum carneae, Pteridietum, Sarothamnetum scoparii, Calamagrostidetum villosae, Juniperetum nanae, Arctostaphyletum, Cytisetum emeriflori.

Espalier shrub, Adpressed shrub, Adpressifruticion. Loiseleurietum procumbentis, Loiseleurietum procumbentis, subass. Vaccinietum uliginosi, Dryadetum octopetalae, Salicetum retusae (?), Hederetum.

Duriprata or **Duriherbosa** and **Sempervirentiprata** or **-herbosa.** *Hardgrass prairie and dry grasslands.* Stipetum pennatae, Festucetum vallesiacae, Festucetum capillatae, Festucetum variae, Brachypodietum pinnati, Calamagrostidetum (?), Xerobrometum erecti, Mesobrometum erecti, Mesobrometum brachypodiosum pinnati, and other subass., Nardetum strictae, Nardetum trifolietosum alpini, Seslerietum coeruleae alpinum, Seslerietum anthyllidetosum, Trifolietum repentis, Caricetum firmae, Caricetum sempervirentis, Elynetum, Curvuletum. *Moist grasslands.* Caricetum ferrugineae, Cynosuretum cristati, "Milchkrautweide" (Leontodontes), "Mutternwiese" (Ligusticetum mutellinae), Caricetum albae, Brachypodietum silvatici, Luzuletum spadiceae, Festucetum violaceae, Caricetum refractae, Calamagrostidetum arundinaceae, Holcetum lanati, Festucetum nigricantis. *"Schneetälchen," snow flush vegetation.* Salicetum herbaceae, Alchemilletum pentaphylleae, Polytrichetum sexangularis, Anthelietum, "Kalk-Schneetälchen" (?). *Arrhenaterion, Fettmatten, manured meadows.* Arrhenateretum elatioris, Trisetetum flavescentis, Trisetetum agrostidetosum capillaris, Trisetetum festucetosum rubrae commutatae, Poetum alpinae, Poetum annuae.

Altherbosa. *"Karflur," Kar herbage.* Epilobietum angustifolii, Adenostyletum, Peucedanetum ostruthii, Cicerbitetum, Petasitetum nivei. *"Läger," lair herbage.* Rumicetum alpini, Senecionetum alpini, Lappulo-Asperugetum.

Emersiprata or -herbosa. *Molinietalia coeruleae. Molinion coeruleae.* Molinietum coeruleae, Molinietum caricetosum hostianae, Molinietum caricetosum paniceae, Molinietum caricetosum tomentosae, Filipenduleto–Geranietum palustris, Scirpeto silvestris-Deschampsietum caespitosae. *Caricetalia fuscae. Rhynchosporion albae.* Caricetum limosae, Rhynchosporetum albae, Rhynchosporetum trichophoretosum alpini. *Caricion fuscae.* Schoenetum nigricantis, Schoenetum eleocharetosum pauciflorae, Schoenetum nigr. schoenetosum ferruginei, Juncetum subnodulosi, Caricetum lasiocarpae, Trichophoretum caespitosi, Caricetum davallianae, Caricetum fuscae, Trichophoretum pumili, Caricetum lachenalii, Caricetum frigidae, Caricetum paludosae, Caricetum incurvae, Juncetum compressi, Caricetum frigidae, Epilobietum alsinifolii, Montietum, Cardaminetum amarae. *Phragmitetalia.* Magnocaricion elatae; Caricetum elatae, Caricetum el. potamosum graminei, Caricetum el. agrostidosum albae, Caricetum el. phragmitosum, Caricetum el. phalaridosum arundinaceae, Caricetum el. caricosum gracilis, Caricetum el. caricosum acutiformis, Caricetum inflati-vesicariae, Caricetum distantis, Eriophoretum scheuchzeri.

Phragmition communis. Scirpeto-Phragmitetum typicum, Scirpeto-Phragmitetum schoenoplectosum lacustris, Scirpeto-Phragmitetum phragmitosum, Scirpeto-Phragmitetum phalaridosum, Scirpeto-Phragmitetum glyceriosum aquaticae, Scirpeto-Phragmitetum typhosum, Glycerieto-Sparganietum neglecti, Glycerieto-Sparganietum negl. potamosum, Glycerieto-Sparganietum negl. juncosum, Glycerieto-Sparganietum negl. epilobiosum. *Litorellion uniflorae.* Eleocharetum acicularis, Isoetetum echinospori.

Submersiherbosa. *Potametalia.* Potamion eurosibiricum, Parvopotameto-Zannichellietum tenuis, Potametum panormitano-graminei, Potametum nitentis, Potametum perfoliati-Ranunculetum fluitantis (subass. potametosum lucentis, subass. sparganietosum), Myriophylleto verticillati-Nupharetum, Ranunculetum flaccidi, Sparganietum affinis, Hippuridetum, Characetum, Nitelletum, Nereids, Hydrocharids, Beggiatoetum, etc.

Sphagniherbosa Sphagneta, Eriophoretum vaginati.

Deserta. *Alluvial ground* (*Alluvialflur*). Myricarietum germanicae, Epilobietum fleischeri. *Arable ground* (*Ackerflur*, *Arvideserta*), Segetalia (Saatkulturen, sown cultures), Agrostemmatetum githagonis, Olitoria (Hackkulturen, hoed cultures), Chenopodietum polyspermi, Ruderalia. *Sliding rubble* (*Geröllflur*). Thlaspeetum rotundifoliae, Petasitetum nivei, (Dryopteridetum robertianae and aculeatae), Allosoretum crispi. *Fixed rubble* (*Schuttflur*), Arabidetum coeruleae, Androsacetum alpinae, Oxyrietum digynae. *Rocky ground* (*Felsflur*, *Rupideserta*). Androsacetum helveticae, Potentilletum caulescentis, Potentilletum caul. kerneretosum saxatilis, Primuletum hirsutae, Androsacetum imbricatae, (Agrostidetum rupestris, Festucetum ovinae). *Stony and woody ground* (*Stein-* und *Holzflur*, *Saxideserta*). Andreaeetum petrophilae, Drepanietum filiformis, Antitrichietum, Lobarietum pulmonaceae, Parmelietum furfuraceae, Parmelietum scorteae, Xanthorietum parietineae, Ephebeetum lanatae, Jonaspetum suaveolentis, Gyrophoretum cilindricae, Ramalinetum strepsilis, Rhizocarpetum alpicolae, Biatorelletum testudineae, Biatorelletum cinereae, Toninietum candidae, Gyalectetum cupularis, Pertusarietum corallinae, Aspicilietum cinereae, Cyanocapsetum, Scytonemetum crassi, (Bacillariacetum Diatomeenschlamm),(Chromulinetum, Leuchtalgenanflüge), (Gloeocapsetum, Endolithen), (Gloeocystetum), (Pleurococcetum, grüner Staub).

Plankton. *Real plankton communities. Kryoplankton*, etc.

Edaphon. *Communities of soil bacteria*, etc.

LIGNOSA

As we have seen, Switzerland shows three great orographical divisions: the Alps; the midland or high plain, 400-500 m. mean level; and the Jura (mountains). Vertically we have to distinguish the following belts: (1) the colline belt or culture belt; (2) the montane belt or deciduous forest belt (Laubwaldstufe): (3) the subalpine or conifer belt; (4) the alpine belt above tree limit (timber line[3]); (5) the niveal belt above perennial snow limit.

[3] "Timber line" is not precise enough, it may include several distinct limits: forest limit (the closed community), tree limit (the single high stem), cripple limit (dwarf forest tree), and wood limit (of any woody species, not tree-like).

The principal limits run at very different altitudes according to the character of climate and the mean elevation of the land (Massenerhebung). Tree limit is at about 1500 m. in the west, rises towards the Alps and reaches 2300 m. in the highest masses of the Engadine and of the "Wallis" (Zermatt). The limit of permanent snow runs at 2400 m. in the Prealps, but reaches 3200 m. in the highest central Alps.

The most abundant broad leaved tree is the beech, *Fagus silvatica*, 83 per cent of all the leafy trees. The beech forests are thus very important and would in a natural condition be much more extensive still.

The beech invaded the country only in early postglacial times and conquered the land from the oaks and firs. *Fagus silvatica* likes medium, somewhat oceanic climate, and rich soil; it thrives in the midlands, especially in the rainy foothills with above 100 cm. annual precipitation. In the Zürich municipal forest in the Sihl valley it seems to be at its optimum, thriving like a weed. With its dense shade it can kill an oak wood in one generation. Our beech forests compare well with the American beech-maple forests, but they cast an even deeper shade, because the shade-giving beech is more dominant; whereas your maples, which allow more light to pass, are more frequent than the maples, lindens, hornbeams, ashes, etc., in our beech forests.

The lower layers of the forest under the dense canopy enjoy a locally more mild oceanic climate. Evergreens are rather abundant, especially on the slopes of the Alps; yew (*Taxus baccata*), holly (*Ilex aquifolium*), and ivy (*Hedera helix*) are frequent. *Anemone nemorosa* is a dominant herb in the midlands, *Allium ursinum*, the bear's garlic, in wetter places. There may be associations of Fagetum anemonosum and Fagetum alliosum.

At higher but still montane elevations *Abies alba*, the silver fir, a species which bears dense shade, is often freely present. Towards the upper limit of beech forests and in the mild oceanic "Föhn" valleys *Acer pseudoplatanus* is a constant and abundant associate.

Towards the south the limits between different belts of vegetation run at higher levels. On the southern slopes of the Alps beech forests rise to 1500 m. and form the tree limit, since no upper conifer belt exists in the insubrian climate of the Sottoceneri. The beech forest, however, is limited below at about 800 m. From this level downwards to the insubrian lakes (for example, Lago Maggiore at 196 m.) there are chestnut forests (*Castanea sativa*) on soils poor in lime; oak forest on calcareous soils where they have not been removed; vine "gardens" (*Pergola*) with corn and vegetables planted between the vines are plentiful. *Quercus sessiliflora* is a light-demanding tree, enough light penetrates to allow a grass sward, so that we may call the association a Quercetum sessiliflorae pratosum.

The chestnut, *Castanea sativa*, occurs in two distinct conditions according to its treatment; as "Selva" or high forest with fine fruit-bearing trees and pasture undergrowth, and as "Palina," a coppice cut every 10–15 years for fuel. Here forest lies at the lowest elevation in Switzerland. The chestnut belt

also rises in going south and forms in the Mediterranean a belt above the basal Macchia belt.

In our midland river beds we have the various "Auenwald" series leading to the beech climax (Siegrist '13). On alluvial sand a most frequent series runs from *Myricaria germanica* scrub through willow and alder wood (mostly *Alnus incana*) to beech forest, on coarser drier material it runs from the sandthorn scrub (*Hippophae rhamnoides*) through the edaphic forest of common pine, *Pinus silvestris*, to the beech climax.

Pinus silvestris, a very unexacting species which occurs everywhere, occupies in the midlands and northern Alps all sorts of habitats not good enough for the climax beech, such as quarries, windy wood borders, dry crests and hills, rubble, or places where the forest soil has deteriorated from bad treatment. But as soon as the soil has improved as a result of occupation by the pine stand, broad leaved trees grow up and kill the pine by overshading.

In the central Alps, where the climate is continental, things are quite different. Broad leaved forest cannot thrive there, the last beeches occur in wet ravines, in sheltered moist places, or in mist belts, but the pine forests form the climax in this region, especially from 600–1500 m. Many of you know Graubünden and "Valais," especially the Engadine and Zermatt, and will have seen pine forests in the Rhine valley from Chur to Dissentis, in the Albula valley from Thusis to Bergün, in Davos, in the lower Engadine, and in the Puschlav, then on the Gotthard route from Erstfeld to Göschenen, and in Faido; also in the Rhone valley and its side valleys. Phytogeographically these areas are called the pine region: it is very rich in interesting xerotherm plants which find a suitable climate to grow in, though most of them grow outside the pine community.

In the broad subalpine belt we have the spruce forest climax occupying extensive areas. In the Jura the belt runs roughly from 700–1500 m., in the northern limestone Alps from 1400–2000 m., where it forms the tree limit. Spruce forests like good, deep soil but extend indifferently through all minor varieties of climate and soil. *Picea excelsa* is a shade tree; it can grow in such close canopy that undergrowth is scanty, especially in the lower areas. Constant members of the ground vegetation are *Oxalis acetosella*, *Hieracium murorum*, *Melampyrum silvaticum*, etc.; under the more open canopies blueberry *Vaccinium myrtillus*. In the moister lower areas, sheltered from frost, *Abies alba* is still an associate. *Picea excelsa* is the most frequent tree in Switzerland, amounting to 40 per cent of the forest trees present, beech amounting to 25 per cent, *Abies alba* 20 per cent, and all other species far less. A considerable amount of spruce is also grown in the beech lowlands on account of its valuable building timber. The percentage of spruce is thus artificially enlarged.

In the continental central Alps there is still another forest community occupying a forest belt above the spruce, and consisting of larch and cembra pine forest. These grow from 1800–2300 m., by preference in the Engadine and Valais. *Larix europaea*, our only deciduous conifer, is strongly light-demanding, so that it cannot compete with spruce where this thrives. Larch can grow

on new mineral ground, it colonises alluvial and fixed rubble. On account of its openness larch forest permits a grassy ground vegetation, and Laricetum pratosum is a frequent association. On the other hand *Pinus cembra*, the cembra pine, the type of the five leaved section of pines, forms beautiful dark green cylindrical trees which give and endure dense shade. The undergrowth is mostly dwarf shrub heath of *Vaccinium myrtillus*, *Vaccinium vitis idaea*, *Rhododendron ferrugineum*, etc. This we call a Pinetum cembrae fruticulosum. Larch and cembra are mostly found mixed; the natural succession tends from larch to cembra wood, but they are also a little divided by altitude, cembra holding the higher belt. On the southern alpine slopes larch forest also occurs immediately above the beech forest whilst farther south, as we have seen, the beech forms the insubrian tree limit, as it does on submediterranean mountains, for example, in Corsica.

Let us pass to *scrub communities*. In the forest belts wherever scrub can grow climatically trees can also grow. Therefore scrub is edaphically or biotically conditioned. Edaphic scrub we have mentioned in the alluvial successions. A biotic scrub is the Coryletum avellanae, a hazelnut bush pasture, where grazing and bush browsing have eliminated tree growth and favored scrub of unlimited sprouting power (Ausschlagsvermögen). On the coarse material of rubble heaps a characteristic association springs up, a briar bramble bush, Rosetum with different species of *Rosa* and *Rubus*, with *Rhamnus cathartica*, characteristic tall herbs, and casual apophytes.

In the subalpine belt we have two very characteristic scrub communities the green alder scrub, Alnetum viriduis, and the prostrate pine scrub Pinetum montanae prostratae (Legföhrengebüsch). Both have flexible ascending branches which may form quite impassable thickets. Alder prefers wet siliceous slopes, mountain pine drier calcareous ones. They may grow in avalanche ravines, on rubbly slopes where trees would be crushed by the avalanche or other descending material, but the flexible branches of the shrubs bend down under the weight of the avalanches and rise again uninjured. There is an old dispute about this scrub as to whether it grows only within the conifer forest belt or also above. In the eastern Austrian Alps a strong pine belt (Legföhrengürtel) exists nowadays above the forests, but probably the forest limit has been depressed by man. The solution seems to be that these scrub associations extend higher than spruce forest but keep within the larch-cembra forest belt.

Dwarf shrub communities. Some Jura scrub, especially the Buxetum of *Buxus sempervirens*, which is so abundant in the Spanish mountains and on the Balearic Islands, shows submediterranean affinities. In the Alps we have extensive dwarf shrub heath of laurel leaved and heath leaved shrubs. The "Alpenroses," *Rhododendron ferrugineum* on siliceous and *Rhododendron hirsutum* on calcareous soil, are dominant, with the blueberry *Vaccinium myrtillus* and the cranberry *Vaccinium vitis idaea*, and heather *Calluna vulgaris*. These communities like the oceanic climate. In the continental central Alps they find an intermediate to oceanic climate locally under the trees and also above the forest belt, as precipitation rises with altitude, at least in this belt. Besides,

this upper belt is more misty than the dry valleys. Markedly characterized by dwarf shrub heath is the ever-wet and oceanic Grimsel pass area in the Bernese Oberland (Frey '22). The "Alpenrose" association lives in the same belt as the larch-cembra forests, and it replaces those forests in areas where spruce forms the timber line. In winter it needs snow covering and you see dead branches wherever the bush emerged from the snow during winter. The dwarf shrub associations of the Alps are very closely related to those of Scandinavia.

To the shrub belt also belong associations of quite prostrate species pressed more or less to the ground. We therefore call them espalier shrub, adpressed shrub, I would name this group of associations Adpressifruticion. There is especially the association dominated by the beautiful alpine Azalea, *Loiseleuria procumbens* with rose colored flowers, often abundantly interspersed with the moorberry *Vaccinium uliginosum*, which may become dominant and thus form a subassociation or consociation. The southern types of dwarf shrubs are mostly "rod shrubs" or "switch plants" and the most frequent dominant is the broom, *Sarothamnus scoparius*.

HERBOSA

Terriherbosa. Forty per cent of the area of Switzerland is grassland, nearly double the area of forested land. This is of course not a natural condition, but results from cattle raising as the principal agricultural practice. Above the tree limit in the alpine belt grasslands are climatically conditioned, but the grasslands below timber line, with the exception of some which are edaphically conditioned, are all due to the biotic, that is, the agricultural factor; regular mowing excludes shrubs and trees.

A first group are the hard grass meadows or hard grasslands, the Duriherbosa. Their ecology is nearly related to American and Hungarian prairies and plains. Mechanically stiffened grasses are dominant, bulbous plants are plentiful. Stipetum pennatae, Festucetum vallesiacae in the dry continental Valais remind us of Hungaria and Russia, a Festucetum capillatae occurs in the Puschlav. In less dry regions such associations are found edaphically conditioned on sunny southern slopes where wood or scrub is excluded by dryness and scanty soil. But nearly the whole of Switzerland's grasslands are evergreen grasslands Sempervirentiprata. Quite half are mowed, a little less than half are pastures, the latter mostly above timber line.

A well defined association group is the Arrhenaterion, the manured meadows (Fettmatten). The biotic factor of permanent manuring and mowing has caused selection to act so well that we have associations with well defined constants and characteristics. In the lower lands it is the Arrhenateretum elatioris, an association dominated by the oat grass, *Arrhenatherum elatius*; large umbellifers, dandelions, and a score of others exhibit different aspects or even different consociations. Mowing is done at least twice a year. In the upper montane and the subalpine belt *Trisetum flavescens* the golden oat prevails, accompanied by its followers; if the ground is a little wetter Trisetetum agrostidetosum capillaris, a subassociation of the common bent grass *Agrostis capillaris* pre-

vails, when a little drier we have the Trisetetum festucetosum rubrae commutatae, the consociation of a variety of the red fescue. Still higher up, the alpine spear grass, *Poa alpina*, dominates an association of this group.

Of the multitude of associations of dry grasslands and moist grasslands I can only mention a few. A very prominent group are the dry meadows of the Brometalia order, so-called after *Bromus erectus*, dominating two associations of the Bromion group, the Xerobrometum and the Mesobrometum. Most of the land naturally belonging to these associations has become arable, bearing triennial rotation of crops. On calcareous soils from the lowlands to the high alpine belt associations dominated by the European blue grass, *Sesleria coerulea*, prevail. Siliceous dry soils are frequently occupied by the Nardetum strictae, the dominant grass of tough bad pasture. A very conspicuous association is the Curvuletum or Caricetum curvulae forming the highest grassland up to and even above snow line, from 2300–3100 m. It is one of the best studied associations, being treated in many monographs and summarized in my paper "Curvuletum" ('22a). The corresponding calciphil association is the Caricetum firmae, with its dominant *Carex firma*.

Moister northern slopes in the montane belt are covered on calcareous soils by the rust sedge association, Caricetum ferruginei, in the subniveal belt by the Luzuletum spadiceae. But one of our most interesting moist meadows is the snow flush, the "Schneetälchen," so called by our veteran master Oswald Heer in 1835, the first well defined plant community. Little dells where the snow lies late, or there is a long continued supply of cold water, and other moist areas where humus accumulates, are always occupied by the same relatively small number of species. These endure long winter covering, they like rich soil, and they are curiously adapted for quick assimilation after the running off of the water covering. Many of the species have hairy or waxy surfaces, leaving the leaf at once dry and ready for assimilation when the water has run off. *Salix herbacea*, *Alchemilla pentaphyllea*, *Polytrichum sexangulare*, *Gnaphalium supinum*, and the liverwort *Anthelia* are conspicuous, *Veronica alpina*, *Cardamine alpina*, *Arenaria biflora* are very characteristic. In the upper alpine belt of cold oceanic Scandinavia these communities represent the climatic climax and cover extensive stretches of land.

Where the soil is very luxuriant, tall herbs form communities called Karherbage from the glacier moulded Kars where these species often occur. Among them are *Adenostyles*, *Aconitum*, *Peucedanum ostruthium*, *Epilobium angustifolium*. Around the alpine dairy huts where the soil is over-manured the rank tall herbs *Rumex alpinus*, *Senecio alpinus*, *Chenopodium bonus henricus*, etc., form the "lair" herbage.

The emerged meadows have been lately treated systematically and critically by Koch in the three community orders of Molinietalia, Caricetalia fuscae, and Phragmitetalia. The areas of broom reed associations dominated by *Molinia coerulea* (Besenried = broom reed) are very much enlarged by the action of man, because these communities furnish very good litter for cattle. The late mowing in fall favors the growth of *Molinia* and keeps the succession in this

stage of wet meadow. Another group of wet meadows or fens or herbaceous marsh center around the common sedge *Carex fusca* (=*C. Goodenoughii*), while the land building types (Verlander) of tall carices group themselves around *Carex elata* (=*C. stricta*), the tall stiff sedge, Kerner's Zsombek formation in the Hungarian Danube area. These communities lead to the regular reed swamp of the cosmopolitan *Phragmites communis*, of the cat-tail *Typha* and the bulrush *Schoenoplectus lacustris*.

Submersiherbosa and Sphagniherbosa. The submerged communities of Nereids and Hydocharids are likewise cosmopolitan as also the whole order of pond weed communities, the Potametalia.

In wet parts of Switzerland in the Jura, near Einsiedeln, etc., we also have *Sphagnum* moors (described by Früh and Schröter '04), similar to, but not so extensive as those in Great Britain and Scandinavia.

DESERTA

I will touch on the vegetation of Deserta in a few words only, though the area of it, that is, of unproductive land, is very large and many of our most beautiful alpine flowers occur here. According to the soil and to the altitudinal belt we have well characterized associations, that is, in siliceous sliding rubble (slipping talus) the Allosoretum crispi, in calcareous the Thlaspeetum rotundifolii; on rocky ground the siliciphil Primuletum hirsutae, the calciphil Potentilletum caulescentis in the subalpine belt, above this the Androsacetum helveticae. Whoever goes climbing in the Alps will surely never forget the subniveal fixed rubble where shine the cushions of dark blue *Gentiana bavarica*, where the pink stars of *Androsace alpina* gleam, *Eritrichium nanum*, the heavens herald, sparkles, and where the glacier crowfoot *Ranunculus glacialis* ascends the peaks up to 4270 m. as the highest flowering plant of Switzerland and of Europe.

INSTITUTIONS AND PERIODICALS

In our little country with its extraordinary richness and variety of scenery and habitat oppotunities for research in geobotany (ecology, plant geography) are abundant and manifold though restricted in material means. Societies with more or less possibility for publishing are the Swiss Botanical Society, a few little botanical societies of the single states (Zürcher Botanische Gesellschaft, Société Botanique de Genève, Berner Botanische Gesellschaft), many individual cantonal associations for the advancement of science in general, and the large Swiss Association for the Advancement of Science—Schweizerische Naturforschende Gesellschaft. Each of them publishes more or less extensive yearbooks, transactions, proceedings, or the like. Eight universities, seven single cantonal (state) universities and one federal technical university (Eidgenössische Technische Hochschule) have botanical institutes, and in most of them ecology takes a more or less prominent place. Though we have no special ecological society, there is now a permanent institution for the advancement of geobotany, the Phytogeographical Commission of the Association for the Advancement of Science. In general, what other countries organize in state

surveys (geological survey, etc.) is done in our country by the several commissions of this Association. That is our Academy of Sciences.

The phytogeographical commission publishes the well known green series: "Beiträge zur geobotanischen Landesaufnahme" (Contributions to the geobotanical survey of Switzerland). The commission also cosiders the regulating of cartography, of geobotanical nomenclature, and so on.

Another institution is entirely devoted to our science, the "Geobotanische Institut Rübel in Zürich" where a herbarium, a library, a collection of ecological instruments such as are described in my book on geobotanical research methods ('22) are at the disposal of anyone who intends to do research work, especially advanced researchers who are no longer connected with a university and its facilities. This institute is constituted as a foundation of public utility (gemeinnützige Stiftung). It publishes the blue series of "Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich" nebst "Beiblättern" (supplements).

I must close. In this short time I could only give a very fragmentary sketch of phytosociological research work in Switzerland, its genesis, its methods, the plant communities it recognizes, its publishing facilities, but I hope you agree that our little country with its restricted means does what it can for our beloved science and I also hope that I may have contributed, for the purposes of our international discussion, some few hints towards facilitating mutual agreement and unification of the methods of research.

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[From an extended "Selected list of literature" prepared by the author on geobotanical research in Switzerland. it has been necessary from practical considerations to select again only those articles referred to in this paper. For the extended list see Beiblatten den Veröffent. d. Geobot. Inst. Rübel in Zürich. No. 4. 1928. Editor.]

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THE FUNDAMENTAL UNITS OF VEGETATION[1]

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One of the most conspicuous features in present plant ecology is the lack of general agreement between different students not only as regards the fundamental problems but also as regards the fundamental units of vegetation and their nomenclature. Though the term "association" now seems to be generally accepted to designate the fundamental units based upon floristic homogeneity, different authors have given the associations a very different extension and are delimiting them in very different ways. The term "formation" is used in different countries to designate units of quite different nature, that is, in continental Europe the abstract types of vegetation units based upon physiognomy, but in England and America the concrete spatial units known in Europe as "vegetation regions." Of course it is indifferent what we name our units of vegetation, so long as we try to give the same type of unit the same name; or, if we cannot come to an understanding, at least we should make the differences in terminology quite clear before we criticize each other. Many of the polemical papers in ecology would have remained unwritten, if these authors had known that they were talking of quite different things, whereas they believed that they meant the same thing, and that only because they used the same term.

The aim of the present paper is to review the different types of fundamental units in plant sociology, to make clear the differences in the use of some fundamental terms in different countries, and to suggest a convenient basis for a more general agreement in future nomenclature.

The fundamental units of vegetation can be arranged in 5 main groups: (1) spatial, (2) taxonomical (systematic), (3) physiognomical, (4) ecological, and (5) temporal units.

I. SPATIAL UNITS

1. *The Association.* An association is a plant community characterized by its essentially homogeneous floristic composition, at least as regards the dominant species, and its essentially homogeneous physiognomy. This definition of the plant association seems now to be generally accepted, though often modified in one or another way by different authors. It leaves ample space for variation in its practical use. Many authors (for example, Clements) use the associa-

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tion concept in a broad sense and divide each association into a great many subordinate units. Others (and among them I include myself) prefer a narrower delimitation of the association.

Two essentially opposite views of the association concept are found in present day plant sociology. According to most continental European and some American authors (for example, Nichols) the associations are merely abstract types, constructed by plant sociologists as a result of comparison and abstraction out of concrete pieces of vegetation constituting the real fundamental units and named "Associationsindividuen," "Lokalassociationen," etc., by European authors and simply "association concrete" by Nichols. According to most English (for example, Tansley) and American (for example, Clements) authors, the associations are concrete units formed by the sum of the mentioned isolated pieces taken as a whole ("the aggregate concept of the association" according to Nichols). Recently, the same view has been put forth on the European continent by Alechin in a very clear and suggestive way.

In my earlier works I have tried to reconcile these two opposite views, admitting the associations to be abstract types though based on concrete realities, that is, by no means merely arbitrary abstractions, as believed by many European authors. After having considered the whole question very thoroughly I think, however, that the only entirely logical treatment is to accept the concrete (aggregate) view of the association without any compromise. Abstract types (like xerophytes, hygrophytes, etc.) are delimited quite independently of the proportions (or relative frequency) of the classified objects, but the delimitation of associations (as well as species) depends to a very large extent upon the lack or relative scarcity of certain transition types forming the limits between the association, thus the extermination of any part of the variational amplitude, or the reappearance of a lacking part of it being quite enough to alter the delimitation of the units considered. According to this view, the association is a concrete part of actual vegetation, though mostly split up into a large number of isolated parts. I am quite unable to regard each of these isolated parts as a fundamental unit of vegetation analogous to the individuals, the real fundamental unit being the sum of all the parts showing essentially the same composition. If the whole association is condensed into a single great continuous piece of vegetation, or if it is split up into several hundreds or thousands of isolated pieces, seems to me to be a matter of quite subordinate importance.

There is no general agreement as to which species should be regarded as most important for the delimitation and the characterization of associations. I think, however, that most sociologists would agree in regarding as of paramount importance the dominant constants. By that I mean the species which are dominant and which constantly occur in every part of the variational amplitude of the association. Moreover, I think the dominants of the different layers should be equally considered. Many authors regard the dominants of the lower layers only as sub-dominants, an opinion I do not share. Thus, I think the *Empetrum-Cetraria nivalis* association and the *Empetrum-Cladonia alpestris* association of

the arctic-alpine heaths are quite distinct associations, *Cetraria nivalis* and *Cladonia alpestris* being quite as important as dominant constants in the ground layer as *Empetrum* in the field layer.

Of the other species of the association I regard the non-dominant constants as most important for the characterization. By non-dominant constants I mean the species which, while not dominant occur very regularly in every part of the variation amplitude of the association. Such species are hardly so important for the delimitation, which should be made chiefly on the basis of the dominant constants of the association, especially when these occur as constants only in the associations concerned. Also those species which are not constant yet confined more or less exclusively to one association (Charakterarten of Braun-Blanquet) are, of course, of some importance for the characterization of the associations, though I think their importance for the delimitation of associations has been much overrated by Braun-Blanquet and his followers.

Most associations, even in the narrow sense used by me, may be divided into minor subordinate units. I think, however, that the time is not yet ripe for a definite fixing of the nomenclature of those subordinate units. Swedish plant sociologists have agreed to name the geographical races of an association facies, and the smaller variations occurring in the same region variants. Contrary to most American and English ecologists, I think it is not convenient to give the same unit different names when occurring as a stable "climax unit" and when occurring as a more labile "seral unit," this being too hypothetical a foundation for such important units. The subordinate units of the association used by Clements and others (consociation, etc.) can hardly be used to designate the subordinate parts of my narrower associations, since in most cases they correspond to what is in my sense the associations themselves; the associations of Clements, on the other hand, corresponding to my subordinate vegetation regions (subregions). The "societies" of English and American authors seem in many cases to correspond to single layers of associations in my sense.

2. *Association complexes.* An association complex is a vegetation unit consisting of an essentially homogeneous mosaic of several associations. Like the association, the association complex is a concrete entity, though often split up into isolated parts. Very fine examples of association complexes are found on high moors (raised bogs), where several associations of dwarf shrub-lichen-heath, dwarf shrub-*Sphagnum* bog, *Eriophorum-Sphagnum* swamp, etc., alternate in very constant proportions in different areas of the surface of the moor. They have been described and very exactly analyzed, especially by Osvald.

3. *Zonation series.* A zonation series is a vegetation unit characterized by a certain zonal arrangement of associations or association complexes. I do not agree with Clements that every zonation series indicates a succession series, this being true on marshy shores, but certainly not on marine and lacustrine rocks, where very fine and regular zonation series occur.

4. *Vegetation regions.* A vegetation region is a major vegetation unit, consisting mostly of a rather variable mosaic of several associations, association complexes, and zonation series, in most cases with some dominant associations,

giving the whole a rather homogeneous aspect. Though many of the associations of a vegetation region stand in successional relations to each other, this is by no means always the case, many associations being quite stable and existing permanently alongside of each other. Practically, the vegetation regions in the sense here applied may be quite identical with the formations of most American authors, notwithstanding the fact that theoretically these are based on supposed successional relations. Thus I think the subalpine birch forest region of Scandinavia should be readily accepted as a good formation by my American colleagues, and similarly should the northern and southern coniferous forest regions of northern Europe, the oak-beech forest region of western Europe, etc. On the other hand, I think the formations of Clements, Shantz, and other American plant sociologists should be regarded as very natural vegetation regions by most European sociologists. As to the concrete nature of the vegetation regions, I think it has been doubted by nobody.

II. TAXONOMIC UNITS

All of the above mentioned units are based upon the actual grouping of species and associations in nature. But like species may be grouped not only according to their spatial relations, into associations, but also according to their taxonomical relations, into genera; associations may be grouped not only according to their spatial relations, into association complexes, vegetation regions, etcs but also according to their taxonomical relations, that is, their similarity a. regards floristic composition, into association groups of different value. Such association groups have been specially emphasized by Braun-Blanquet and his followers. I have found such grouping very useful as a complement to the grouping of associations into formations in the European sense, that is, for the subordinate arrangement of associations within the greater formations. Its usefulness as a substitute for other classifications now generally used does not appear to have been demonstrated.

III. PHYSIOGNOMICAL UNITS

1. *Life forms.* A life form (or perhaps better, vegetation form) is an abstract plant type based upon physiognomy, that is, upon the essential form of their vegetative development. Many systems of life forms have been suggested by different authors, but none has as yet become generally accepted. I will not suggest a new system here, but wish only to emphasize that I think a good one could easily be made by combining the systems of Warming, Raunkiaer, Gams, Clements, and others, with special regard to actual vegetation and to all seasonal aspects.

2. *Formations.* A formation (in the continental European sense) is an abstract type of associations based upon physiognomy, "a life form of associations." Nichols, who has given a very clear account of the different types of vegetational units, names them simply "association types." I think ecologists of our time agree that such types should be based entirely upon physiognomy, not upon a mixture of physiognomy and ecology, as was the procedure of Warming and other earlier authors. Without entering into a further discussion of this question,

I wish only to emphasize that a good system of formations should be based equally upon all seasonal aspects and upon all layers of vegetation. Thus I think that the length of the vegetation period of deciduous species is of paramount importance for the delimitation of formations, and that the field and ground layers are of quite the same importance as the tree layers. The overrated importance of the tree layers seems to me to be the greatest weakness in the formation system established by Brockmann-Jerosch and Rübel.

Of course, all of us are desirous of finding a way to general agreement in the use of the term formation, the present divergence in its use in different countries leading to confusion and quite needless polemic. Whether we bring the continental European or the Anglo-American sense to general application is of minor importance; what is essential is that we should no longer be divided between both usages. I wish, however, to mention that the continental European sense is in closer agreement with the original use of the term by Grisebach than the Anglo-American application.

IV. ECOLOGICAL UNITS

Ecological units (in the narrowest sense), that is, abstract types of associations (or other spatial units) based upon the resemblance of their habitats, such as halophytic communities, xerophytic communities, alcaline communities, etc. Such units have often been confused with the physiognomic units mentioned above. To keep these unit types distinct from each other is, according to my belief, the only way to discover the real correlations between physiognomy and habitat.

V. TEMPORAL UNITS

Temporal units (series), that is, series of associations (or other spatial units) standing in successional relation to each other. I think the same thing should be said of these units as of the ecological units just mentioned, namely, that they should be kept quite distinct from the other types of units mentioned above, because far from putting the successional point of view aside, it is the only way to find the sure successional relations of spatial as well as of physiognomical and ecological units. A detailed account of the different successional units now distinguished is superfluous here, since these units have been so admirably treated by modern American and English ecologists that I would have very little to add. I wish only to say that I think succession and development should be kept more distinct from each other than is done by most authors. When a drier community succeeds a wetter one, in a swamp, this is succession, but not development. When we try to follow the history of present plant communities back to tertiary and earlier periods, finding, for instance, that our present deciduous forests are direct descendants of the tertiary forests, we are studying true development. In the study of this true development, or paleoecology, in the sense of Clements, I think we have the key to many of the most fundamental problems regarding the structure of present plant communities.

PLANT ASSOCIATIONS AND THEIR CLASSIFICATION[1]

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A paper which should compel the thought of every serious student of vegetation has recently been published[2] by my good friend Dr. H. A. Gleason. Under the title, "The individualistic concept of the plant association," Dr. Gleason points out various objections to our current ideas regarding the nature and classification of plant associations, stressing in particular the numerous discrepancies between fact and theory which are all too apparent to most field ecologists. He ends by arriving at certain conclusions which are avowedly heretical regarding our orthodox concepts of the plant association.

In the course of his introductory observations, Dr. Gleason remarks that "The numerous conclusions in synecology which depend directly upon observation or experiment are in the vast majority of cases entirely dependable. . . . But," he continues, "our various theories on the fundamental nature, definition, and classification of associations extend largely beyond the bounds of experiment and observation and represent merely abstract extrapolations of the ecologist's mind. They are not based on a pure and rigid logic, and suffer regularly from the vagaries and errors of human reason." The paper under discussion I am inclined to judge in the light of these remarks. In other words, while there can be no question that the facts herein presented, for the most part, are essentially accurate, the same can not be said of certain deductions which have been drawn from the facts, because many of the facts which Dr. Gleason has set forth in support of his own contentions would seem equally capable of a very different interpretation from the one which he has elected to give them.

Dr. Gleason is in agreement with ecologists generally in recognizing the reality of the plant communities commonly designated associations. As he expresses it: "Plant associations exist; we can walk over them, we can measure their extent, we can describe their structure in terms of their component species, we can correlate them with their environment, we can frequently discover their past history and make inferences about their future. But," he goes on to say, "it seems that we are treading upon rather dangerous ground when we define an association as an area of uniform vegetation, or, in fact, when we attempt any

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 18, 1926. Contribution from the Osborn Botanical Laboratory.

[2] Gleason, H. A. The individualistic concept of the plant association. Bull. Torrey Club 53: 7–26. 1926.

definition of it." According to his ideas, the vegetation of any area is to be regarded as "merely the resultant of 2 factors, the fluctuating and fortuitous immigration of plants and an equally fluctuating and variable environment." The existence of a plant association, he states, depends "solely on the coincidence of environmental selection and migration over an area of recognizable extent and usually for a time of considerable duration." The plant association represents simply "the visible expression, through the juxtaposition of individuals, ... of the result of causes in continuous operation." The 2 "primary causes, migration and environmental selection, operate independently on each area, no matter how small, and have no relation to the process on any other area." Their effect, therefore, is "not to produce large areas of similar vegetation, but to determine the plant life of every minimum area. The recurrence of a similar juxtaposition over tracts of measurable extent, producing an association in the ordinary sense of the term, is due to a similarity in the contributing causes over the whole area involved." In his opinion, therefore, the plant association can scarcely be accorded even the rank of a vegetation unit, to say nothing of being compared with an organism: it is "merely a coincidence."

Now it must be conceded, as Dr. Gleason has emphasized, that there are wide differences of opinion among ecologists when it comes to the precise definition of the term plant association; but so also are there differences of opinion among taxonomists when it comes to the precise definition of the term species. As with the species concept also, there may be even greater differences of opinion when it comes to the application of the association concept to concrete areas or types of vegetation. But those ecologists are few and far between who would not recognize the plant association concept as something which is at least susceptible to more or less definite characterization. By most ecologists the association is described, more or less specifically, as a plant community characterized throughout its extent by essential uniformity in physiognomy and ecological structure, and by essential uniformity in floristic composition, at least with regard to the predominant species. Essential uniformity of habitat is likewise regarded as characteristic of the association, and by many this is included as a part of the definition. By some ecologists, the term association is applied to the concrete pieces of vegetation which we study in the field, and which correspond to the individual plants[3] of the taxonomist. By others, these individual pieces of vegetation are regarded merely as examples of an association, in much the same way that different individual plants of the same kind may be regarded as examples of a particular species.[4] By many, the term association is applied in both (or in all three) of these senses: it is recognized as applicable both to the

[3] See further remarks on this point later.

[4] Or they are regarded as pieces or parts of a large whole, the complete association *strictu sensu* being looked upon as comprising all the individual pieces of vegetation of a particular kind in existence, taken collectively. From a practical point of view this concept of the association as a concrete aggregate of plant communities is scarcely to be distinguished from that in which the association is treated as an abstract unit.

abstract vegetation-concept and to the concrete individual pieces of vegetation upon which this concept is based. This is a point of view upon which I have elaborated at some length in a former publication[5] and one which I have ascertained, by extended correspondence, meets with favor among a majority of American ecologists and not a few European.

These, then, are the concepts of the plant association which Dr. Gleason holds up to criticism—the concepts by which we recognize vegetation as being built up of a series of vegetation-units comparable, in a way, with species and, like the species, capable of being variously coordinated and classified. These are the concepts which he would supplant with his own so-called "individualistic concept" of the plant association. Dr. Gleason's indictment can not be dismissed too lightly, since he has had a wide and varied experience, extending over many years, in the study of plant communities. It is indeed well worth our while to consider briefly the validity of some of his criticisms, in the light of the facts upon which he bases them, and to ascertain, if possible, to what extent he has proven his case. And, in this connection, it will be instructive to draw certain comparisons between the association concept and the species concept; for the association concept, as just set forth, is certainly analogous, in its relation to the systematic study of plant communities, to the species concept, in its relation to the systematic study of plants.

"We all readily grant," writes Dr. Gleason, "that there are areas of vegetation, having a measurable extent, in each of which there is a high degree of structural uniformity throughout, so that any 2 small portions of them look reasonably alike. Such an area is a plant association. . . . ." "But," he continues, "more careful examination of one of these areas will show that the uniformity is only a matter of degree, and that 2 sample quadrats with precisely the same structure can scarcely be discovered. Consequently, an area of vegetation which one ecologist regards as a single association may by another be considered as a mosaic or mixture of several, depending on their individual differences in definition. . . . . The essential point is that precise structural uniformity of vegetation does not exist, and that we have no general agreement of opinion as to how much variation may be permitted within the scope of a single association." The statement just quoted is undeniably accurate. Without question it would be highly desirable if a general agreement might be reached among ecologists as to the exact scope of the association. But is such agreement essential? Is not the situation here identical with that faced by the taxonomists, one of whom would differentiate into several species what another would treat as a single one? Is the absence of general agreement in the matter of allowable variation any more reason for discarding our concept of the association as a vegetation unit than it is for discarding that of the species as a floristic unit?

A second objection brought forward by Dr. Gleason has to do with the definition of the association in terms of character species. This method, we are

[5] Nichols, G. E. A working basis for the ecological classification of plant communities. Ecology **4:** 11–23, 154–179. 1923.

told, may be quite feasible in "northern latitudes, and particularly in glaciated regions, where . . . . there is a wide diversity in environment and a comparatively limited number of species in the flora. . . . . But in many parts of the tropics, ... where the flora is extraordinarily rich in species, such a procedure is impracticable or even impossible.". Now the situation in the tropics is admittedly complex; and yet it is very doubtful if the complexity is quite so extreme as might be inferred from Dr. Gleason's paper, when he states that "a single hectare [in a tropical forest] may contain a hundred species of trees, not one of which can be found in an adjacent hectare." In this connection, some recent observations by Dr. H. N. Whitford[6] are of interest, since probably no American ecologist is more familiar, from actual experience, with the forests of the tropics. Dr. Whitford writes as follows. "I do not know of a single instance where quantitative measurements have been taken of the tree population in the tropics and where this statement [the one just quoted] would hold true, unless it be 2 plots, each of which presents radically different environmental conditions. I have taken many measurements of the tree population in various places in the tropics. I recall one instance in the Philippines, where strip surveys were made in a forest covering 30 square miles and where practically every hectare measured showed a repetition of the same species. They were mixed, to be sure, with a large number of other species which occurred much more rarely; but even such species, upon careful examination, were usually found to be represented by small specimens in the adjacent hectares. To the average botanist entering the tropical forest," concludes Dr. Whitford, "all seems confusion," and yet, "with a knowledge of the species and quantitative studies of the composition, tropical forests can be classified by associations and can be designated by the generic and sometimes the specific names of the predominating trees." Dr. H. L. Shantz states that he has never found an area such as Dr. Gleason mentions in any region that he has visited.[7] In my own opinion, and from a purely ecological point of view, as I have expressed it elsewhere,[8] the situation which Dr. Gleason has in mind may be explained somewhat as follows: that in the humid tropics the influence of physiographic conditions on plant life tends to be overshadowed by that of congenial climatic conditions, with the result that vegetation practically everywhere, regardless of the nature of topography or soil, tends to merge into one great association or association-type. In other words, the uniform climatic climax, toward which the vegetation in every region tends to progess, may come much closer to actual realization in the humid tropics than in temperate regions, where its

[6] In letter to the author.

[7] In this connection, Dr. Shantz calls attention to figures by Bertin, based on the survey of tropical rain forests in three widely separated regions of western Africa (see Shantz, H. L., and Marbut, C. F. The vegetation and soils of Africa. New York, 1923), which indicate a remarkable degree of similarity in taxonomic composition, with respect to predominant trees.

[8] L. c. (Ecology **4**: 1923).

attainment is very largely prevented through the disturbing influence of varying physiographic conditions. Taking into consideration all of these various circumstances, therefore, it is only to be expected that the associations of the tropics should be exceedingly complex, and that they should be correspondingly difficult to interpret and to characterize.

Proceeding further, Dr. Gleason concludes that "environment can not be used as a means of defining associations with any better success than the vegetation." For one thing, he says, "different areas of what are generally called the same association do not always have precisely the same environment." The question may well be asked: Do they ever have precisely the same environment? It is very doubtful. Is there any reason why they should? Certainly not, when we take into consideration the more or less wide range of environmental conditions to which practically every plant, and therefore practically every community of plants is adapted. For another thing, says Dr. Gleason: "Two environments which are identical in regard to physiography and climate," but situated in different parts of the world, "may be occupied by entirely different associations." Surely no one would expect 2 such environments, situated in regions which are floristically different, to be occupied by anything but different associations. Dr. Gleason's point is well taken if what he means is that the association cannot be defined in terms of environment alone. His criticism does not necessarily detract, however, from the usefulness of habitat uniformity as a criterion, where this is used in conjunction with other characteristics derived from the vegetation.

Again, "it is not always possible," writes Dr. Gleason, "to define with accuracy the geographical boundaries of an association," and in the transition zone between 2 adjoining associations there may occur various mixtures between the two. Reverting to our analogy between the association concept and that of the species, is not a piece of vegetation which comprises a mixture of the characteristics of two associations quite comparable to a plant which exhibits a mixture of the characteristics of two species? If we refuse to recognize the plant association as a vegetation unit on this account, are we not almost equally justified in refusing to recognize the species as a floristic unit because of the existence of hybrids?

We read further that "Species of plants usually associated by an ecologist with a particular plant community are frequently found within many other types of vegetation." This is indisputably true. It is a condition which usually can be explained by the wide range of environmental tolerance in the plants concerned, by the recognized absence of absolute environmental uniformity in the areas occupied by any particular association, or by various phenomena associated with succession. But even when these irregularities take the form of so-called "fragmentary associations," is there any occasion to be seriously disturbed? In comparing the association, as a vegetation unit, with the species, as a floristic unit, do we not recognize that the association, by its very nature, represents a unit of a much more primitive order, and one which is much less

susceptible to exact delimitation? Even in taxonomy, for that matter, it is a familiar observation that various structural peculiarities usually associated by the taxonomist with one particular species are frequently found in many other species of plants. The circumstances in the two cases are of course not strictly parallel, and yet they seem sufficiently so to warrant a comparison which is at least suggestive.

Another argument that is advanced by Dr. Gleason against recognizing the association as a vegetation unit, comparable with the species, is the fact that an association varies more or less in its vegetational structure from year to year. "Slight differences in temperature or rainfall or other environmental factors," he writes, "may cause certain species to increase or decrease conspicuously in number of individuals, or others to vary in their vigor or luxuriance." Apropos of this argument, one can only wonder what would happen to the species concept if its validity were contingent on an absence of variation from year to year. Can any more striking variation within any particular association be imagined than that which exists within many species—the difference, for example, between the alpine and lowland forms of certain species or, in the case of certain aquatic plants, between submerged and terrestrial forms? By way of illustrating his point, Dr. Gleason cites a single concrete example, namely, "the remarkable variation in size of an *Amaranthus* in the Death Valley, which was 3 meters high in a year of abundant rainfall, and its progeny only a decimeter high in the following year of drought." Is this variation in *Amaranthus* any more significant in its bearing on the identity of the particular association, of which the plant in question is but a single component, than it is on the identity of the plant itself as a particular species?

The impracticability of defining the association is still further enhanced, in Dr. Gleason's opinion, by the limited period over which any particular association may endure. "Sooner or later each plant community gives way to a different type of vegetation," and some may exist only for a very brief period of time. Here again we are faced by a fact which is familiar to all students of vegetation. It is a situation which may and does introduce difficulties in the application of the association concept to concrete cases, but it is not one which in any way invalidates the concept itself.

Another criticism which Dr. Gleason directs at the concept of the plant association, considered as an entity or unit of vegetation, and one upon which he lays great stress, has to do with the so-called "repetition of associations on different stations over a considerable area,"— the phenomenon upon which, as he says, "depend our numerous attempts to classify associations into larger groups." This idea, he states, "if carried too far afield, is found to be far from universal." In his opinion, for example, "No ecologist would refer the alluvial forests of the upper and lower Mississippi to the same associations." In passing from the one region to the other there is "an almost complete change in the flora" of these forests, and yet, so gradually do these changes take place, that "there is no place along their range where one can logically mark the boundary

between them." One association merges by imperceptible degrees into the other without any apparent transition zone. Here again, it seems to me, we have a situation which is closely parallelled in the field of taxonomic botany. No one questions the validity of the species as the working unit of taxonomic botany and as the practical basis of plant classification; and yet, there is all manner of discrepancy when it comes to the diagnostic uniformity and exclusiveness of the groups of plants to which the term species is actually applied. In particular there are groups of closely related plants which show a more or less definite tendency to be segregated into 2 or more minor groups, between which are to be found various intermediate conditions. In the case of 2 such minor groups, to quote Hitchcock,[9] "Botanists will differ as to whether we have 2 species with intermediate individuals, one species with 2 varieties or subspecies, or one variable species." It seems hardly necessary to cite examples. The point at issue is simply this: that in the systematic study of plant communities, intergradation and an absence of sharp lines of demarcation are to be expected, even more than in the systematic study of plants. The existence of this condition no more invalidates the concept of the association as an entity or unit than does the corresponding discrepancy in taxonomic botany invalidate the generally accepted concept of the species.

After all is said and done, however, I am strongly inclined to suspect that Dr. Gleason's views, in reality, are not so radically at variance with those held by other field ecologists as might appear. This is true, at any rate, up to a certain point. Practically everyone will agree with him in his three fundamental theses, as first enunciated several years ago,[10] namely that "All phenomena of vegetation . . . . depend upon the phenomena of the individual plant;" that "The plant population of any area is determined by environmental selection of immigrants from the surrounding population;" and that plant associations originate through "similarity of environmental selection and of available sources of immigration." In so far as this last thesis is effective, the association may not inappropriately be designated, in the words of Dr. Gleason, as "a coincidence." But to stop short at this point is to block the pathway to any further progress. Most ecologists will agree with Dr. Gleason that associations never exhibit precise structural uniformity; that they cannot be precisely characterized; that, more often than not, they are incapable of precise delimitation in terms of either space or time; and that the association concept itself is incapable of precise and rigid definition. They would concede that any comparison between the association and the species is purely in the nature of an analogy, and that the likening of the association to an organism must necessarily be taken, very largely, as a figure of speech. In insisting, however, that the association can scarcely be regarded even as a vegetation unit and that there is no method by which associations may be classified into any broader groups, it would appear as though Dr. Gleason had forced his conclusions a bit too far.

[9] Hitchcock, A. S. Methods of descriptive botany, p. 8. New York. 1925.

[10] Gleason, H. A. The structure and development of the plant association. Bull. Torrey Club **44**: 463–481. 1917.

It may perhaps be true, as he says, that "a logical classification of associations into larger groups, or into successional series, has not yet been achieved;" but it cannot be denied that various attempts at such classification have been productive of some very suggestive results. It may well be, as Dr. Gleason metaphorically suggests, that classification in itself consists in little more than the arrangement of our knowledge in pigeon-holes; but it is not desirable that our knowledge should be so organized if we are to make the best use of it?

My own ideas regarding the nature and classification of plant associations, briefly stated, are somewhat as follows.

To begin with, the vegetation of the earth's surface, viewed from the standpoint of plant sociology, may be looked upon as a vast mosaic of different plant communities. In so far as the ecological characteristics of these communities are concerned, both their nature and their distribution in space are determined in large measure by environment. The environmental conditions of the present, from an historical point of view, must be looked upon, to a very high degree, as a heritage from the past. They represent the cumulative effect of processes and phenomena which not only have originated in the past but some of which have long since ceased to operate. In considering the origin and development of particular plant communties and the successional relations of different communities, this historical aspect of the environment is of paramount importance. It is, indeed, an aspect which can not be wholly ignored in any study having to do with the ecological relationships of plant communities. But when it comes to the practical study of these ecological relationships in the field, we must have some definite starting point, some solid foundation of observable facts upon which to place our feet; and these facts, whether we understand them or not, are supplied by the plant communities of the present, on the one hand, and by the environmental conditions of the present, on the other. In other words, no matter what the point of view from which we may elect to carry on our studies, it is the conditions of the present, in the main, that furnish us both the materials and the reactions, and it seems imperative that these should be clearly understood before proceeding further. To this end, it seems to me that, in enabling us to adequately orient our facts and to outline our problems, definition and classification, in plant sociology as in other fields of knowledge, are indispensable.

The fundamental unit of ecological plant sociology is the plant association. For our present purpose, we will consider the association, first of all, as a piece of vegetation, occupying a definite piece of ground and having more or less definite spatial boundaries. No matter what conclusions we may ultimately arrive at regarding the general nature of plant associations, it is these concrete pieces of vegetation with which we start. These are the communities which we actually study in the field and upon which we base our various deductions. These are the communities which many of us are disposed to designate as entities in their relation to the general mass of vegetation. These it is, primarily, whose structure we attempt to diagnose, whose nature we attempt to char-

acterize by definition, which we attempt to correlate with various conditions of environment, and which we even venture to classify.

Technically speaking, however, not all pieces of vegetation of this description are plant associations. In order to be so regarded, a community must exhibit structural uniformity of a certain more or less definite sort. On this point practically all ecologists will agree. They will also agree, as Dr. Gleason has suggested, that there is no such thing as absolute uniformity in vegetation; that in its application to vegetational structure the idea of uniformity, in general, must be interpreted very liberally; that uniformity of any description in the structure of vegetation at best can be only approximate. With these limitations in mind, we may characterize the plant association as a piece of vegetation which exhibits essential uniformity in two things, namely, floristic composition and ecological structure. The term "ecological structure" is one that I have used to include all features of a plant community which are of ecological importance. The physiognomy of a plant community, for example, is but one expression (the most obvious one, to be sure) of its ecological structure.

The definition of the plant association just given, or a similar one, meets with the approval of most ecologists,—provided each be permitted to place his own construction upon what is meant by "essential uniformity." This idea is one which some would interpret quite strictly; others very liberally. Such difference of opinion is unavoidable and of comparatively little moment. Again, some ecologists would interpret the idea of essential uniformity quite strictly in its application to ecological structure but very loosely in its application to floristic composition. They would maintain that variations in floristic composition are of little consequence, from an ecological standpoint, when not correlated with variations in ecological structure, and that the recognition of different associations on the basis of floristic dissimilarities alone is of doubtful ecological value. This point of view is one which, at first thought, would seem quite justifiable. In so far as the association is to be taken as the basis for purely ecological studies of vegetation, it is a point of view which I myself, in theory, should feel inclined to follow. When it comes, however, to the practical application of this idea to concrete cases, there is one aspect of the matter which cannot be overlooked, and that is the great deficiency in our knowledge concerning the exact ecological significance of various peculiarities in floristic composition. There can be no question that every species of plant possesses not only a certain more or less definite taxonomic identity but also a more or less definite ecological individuality. This being true, it follows that any peculiarities in the floristic structure of plant communities may be of ecological as well as taxonomic import. Without question the ecological significance of many floristic pecularities of vegetation is negligible; but, on the other hand, we know as a fact that certain peculiarities in floristic composition, even when not accompanied by manifest peculiarities in ecological structure, frequently indicate peculiarities of vegetation which are of unquestioned ecological value.

Between these two extremes there are, of course, all sorts of intermediate conditions. My point of view, in brief, would be simply this: (1) that floristic uniformity may be significant in indicating uniformity or lack of uniformity in the ecological structure of vegetation, (2) that, in so far as this is true, or even in so far as there seems a possibility that it may be true, so far, at any rate, is it imperative that floristics should be taken into account by the ecologist. In characterizing the plant association as a piece of vegetation possessing essential floristic uniformity, therefore, we are characterizing it in terms which may be of ecological as well as floristic importance.

Proceeding further, to what extent is a plant association to be regarded as an entity? To what extent may it be likened to an organism? The answer to the first of these questions seems altogether too apparent to require discussion. Considered in its relation to the total mass of vegetation which occupies the earth's surface, any piece or area of vegetation which stands out as distinct from other pieces or areas of vegetation may be looked upon and treated, in its entirety, as an entity. This observation holds true for the plant association, in so far as this is viewed as a concrete piece of vegetation. It must of course be granted that there is a very wide range in the degree of exactness with which different individual associations of this sort can be measured and mapped, that there are all sorts of intergrading conditions between different associations, and that various perplexities are encountered when it comes to the application of the association concept to particular pieces of vegetation. But this is inevitable; for the association manifestly can not be regarded as an entity of the same clear-cut type as a plant or an animal.

An association may even be looked upon as an organic entity, in this sense, if no other, that it is made up of living organisms. The comparison of the association to an organism, however, even as a simile, is not one which is capable of application indiscriminately to all types of association. Viewed as a concrete piece of vegetation, any association may be regarded as an entity, or even as an organic entity; but the only associations which may at all reasonably be likened to organisms are those in which there is active commensalism; whose constituent plants, in varying degree, exhibit the phenomena of competition, priority, dependence, and mutuality.[11] These relationships are most highly developed in climax communities. The comparison to an organism can not logically be applied to primitive communities of scattered plants, in which the various individuals exist in virtual independence of one another, drawn together solely by the physical peculiarities of their common environment. The difference between the association considered as an entity and the association compared to an organism is essentially the same sort of difference as that between succession and development, as pointed out by Tansley.[12]

[11] See Yapp, W. H. The interrelations of plants in vegetation and the concept of "associations." Veröffentl. Geob. Inst. Rübel in Zürich **3**: 684–706. 1925.

[12] Tansley, A. G. The classification of vegetation and the concept of development. Jour. Ecol. **8**: 118–149. 1920.

Coming, finally, to the question of the classification of plant associations, it should be emphasized, first of all, that classification of any description consists in little more than the systematized organization of facts. Whether we call it by that name or not, it is only by means of classification that we can hope to produce order out of chaos. The principle of classification, in the words of Hugh Miller,[13] is one "which we find pervading all science, ... and without which all knowledge would exist as a disorderly and shapeless mass, too huge for the memory to grasp, and too heterogeneous for the understanding to employ." With most ecologists, classification represents merely a means to an end. With a perverted few of us, the methods of ecological classification, in themselves, have become more or less a subject of special study. In the ecological classification of plant associations, the subjects of classification are found in the concrete pieces of vegetation to which, up to now, we have applied the term association. For convenience, these concrete pieces of vegetation hereafter will be referred to specifically as stands,[14] the term association being given a somewhat broader interpretation. As will be apparent presently, the stand holds very much the same relation to the association, considering this in its larger sense, that the individual plant, in the field of taxonomic botany, holds to the species.

Plant associations may be classified in their relation to environment from three different points of view. First of all, the various individual stands which go to make up vegetation may be classified with reference to their inherent characteristics of composition and structure, in essentially the same fashion that plants are classified by the taxonomist. Different individual stands which resemble one another in floristic composition may be referred to a common association. The association, viewed in this light, becomes an abstract vegetation unit of which the stand is the concrete example[15] (the "association concrete," as I have designated it elsewhere[16]). Proceeding further, different individual stands which resemble one another in physiognomy and ecological structure, regardless of any floristic similarity or dissimilarity, may be referred to a common ecological association-type (or formation, as it is termed by many). The individual stand thus affords the material basis for the recognition of abstract vegetation units of 2 kinds, namely the association and the association-type. The relationship between the individual stand (the basic vegetation entity, so to speak), the association (considered as a vegetation unit) and the association-type, in the ecological classification of plant communities, is essentially similar to that existing between the individual plant, the species, and the genus in the taxonomic classification of plants. The importance of the association-type as an ecological unit of classification is generally recognized. That of the association, considered as an abstract vegetation unit, is not so universally

[13] The old red sandstone, ed. 7, p. 80. 1860.

[14] See Christophersen, E. Soil reaction and plant distribution in the Sylene National Park, Norway. Trans. Conn. Acad. Arts and Sciences **27**: 471–577. 1925.

[15] But in this connection see footnote regarding aggregate concept of association.

[16] *L. c.* (Ecology **4**: p. 17, 1923).

accepted. As a matter of fact, the ecological value of the latter, as distinguished from the association-type, depends entirely upon the ecological value of the floristic peculiarities by which it is characterized; and this, too often, is an unknown quantity. As with the genus and species, a particular association-type may include many associations or but one; while the number of associations recognized by different ecologists will vary according to the point of view and the ecological significance which is placed on various features of floristic composition.

A second basis for an ecological classification of plant communities is afforded by their geographical relationships. The ecological characteristics of any particular stand are determined primarily by the local geographical features of the area which it occupies. The distribution of different associations and association-types over the face of the earth is determined in large measure by the distribution of various features of climate and physiography. Different individual stands tend to be grouped into more or less definite association complexes, the nature and limits of which are correlated with various geographical features of the terrestrial environment. Considered from this point of view, different individual stands may be classified as components of various geographically determined association-complexes or geographical plant formations: of formations determined by climate, on the one hand; of formations determined by physiography, on the other. Every stand may be looked upon as a member of some particular climatic formation, and also as a member of some particular physiographic formation. From the standpoint of geographical synecology, the climatic formation and the physiographic formation thus represent vegetation entities of a higher order than the stand.

Of the 2 methods of classification just outlined, the first is subjective, being based upon the ecological characteristics of the vegetation itself. The second is objective, the plant communities being considered in their relation to certain specific features of their terrestrial environment. In one particular, the point of view is alike in both cases, namely, in that it has to do primarily with the conditions of the present day. A third basis for an ecological classification of plant communities is afforded by the phenomenon of succession. From the successional point of view, the plant communities of the present are considered in their relation to the plant communities of the past, on the one hand, and to those of the future, on the other. The viewpoint is essentially that of genetics. Each individual stand may be looked upon as a member of some particular successional series: it is the contemporaneous representative of the series, and as such it may be classified in its relation to the origin and cause, the trend and the climax of the series as a whole.

I have outlined very briefly 3 different points of view from which the ecological relationships of plant communities may be interpreted. Each one of these is logically distinct from either of the other two. Any one of them, by itself, may be taken as a rational basis of ecological classification. All three have actually been so used by various ecologists. In conclusion, it should be borne in mind that, in attempting to classify the facts of nature, we are dealing

with "merging phenomena." Here, therefore, as in all such cases, "the only possible procedure is to select the extreme marked types of the groups and, giving these careful study and description, to describe the intermediate kinds according to their positions between the types."[17] No system can be devised for the ecological classification of plant communities which is absolutely automatic and mathematically precise in its practical applications. Furthermore, the application of abstract ideas of any description in the classification of plant communities can be successful only in so far as we possess a complete knowledge both of the facts and of the principles involved. If our knowledge concerning either of these essentials is incomplete, differences of interpretation are inevitable, no matter what our basis of study. After all, the best that any scheme for the classification of plant communities can hope to do is to furnish a sound framework, based on generalized facts and fundamental principles.

[17] Quotation from Ganong, W. F., in Bull. Nat. Hist. Soc. New Brunswick **5**: pp. 50, 51, 1903.

PLANT ASSOCIATIONS AND THEIR CLASSIFICATION: A REPLY TO DR. NICHOLS[1]

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Dr. George E. Nichols, who this morning read before you a most stimulating discussion of plant associations and their classification, generously allowed me, before the presentation of his paper, an opportunity to examine his manuscript and to prepare a rebuttal. His remarks to you fall into two divisions: the first, a criticism of my own opinions as expressed in a recent printed statement, and second, a continuation of his valuable and well-known ideas on the fundamental nature and classification of plant associations.

To the uninitiated, it might seem that Dr. Nichols and I are fundamentally opposed. Such is by no means the case. Both of us agree absolutely on the existence of those well-known units of vegetation, the plant associations; both of us agree on the importance of a clear understanding of their nature, of the fundamental causes which bring them into being; both of us are sincerely interested in advancing and clarifying ecological knowledge, a science to which both of us have given much of our time and ability for many years.

In his criticism of my views, Dr. Nichols has stated them with a degree of accuracy and fairness which is seldom found in a critique. Those of you who listened to him this morning probably understand my opinions nearly or quite as well as if I had presented them personally. There are, however, various sections to which he has not objected at all, and which appear to me as of some importance. I have accordingly distributed separates of my article to a number of you, and a few are still available to others who may wish to examine it more closely.

My own opinions are the growth of many years. Brought up under the influence of Cowles' early work on succession, and of Clements' hard-and-fast crystallization of ecological phenomena into fixed and inviolable laws, I was originally perfectly able to see the association as an organism, passing by succession through a process of evolution. Gradually these ideas changed, as my experience increased in time and breadth, until now the plant association impresses me as the contemporaneous result of two fluctuating causes, subject to continuous variation both in space and time.

Only one feature of Dr. Nichols' discussion of my views meets with objection. He has repeatedly compared the association with a species. I quote:

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 18, 1926.

"the association concept, as just set forth, is certainly analogous, in its relation to the systematic study of plant communities, to the species concept, in its relation to the systematic study of plants."

Dr. Nichols well chooses the word "analogous" rather than "homologous," since the idea of any homology between the two units is no longer current. An association is not a species of vegetation. Nay more, the differences between them are so fundamental that even the word analogous can not be used with entire correctness. I readily admit that there are similarites between the two concepts, as they exist in the human mind, particularly when we select the concept of the association as held by Dr. Nichols, but that is as far as the similarity extends. Under any concept, a species is a group of individuals the members of which are theoretically related by genesis, and capable of classification genetically when our knowledge is sufficient. But an association has no powers of reproduction. It is merely continued by the reproduction of individual component plants.

Species, also, are supposed to change by evolution into other species, and thus to show an interspecific genetic relation. Not so with associations: the nearest approach to such a process is found in succession, and in that phenomenon the original species disappear, new arrivals appear from some outside source, and there is no evolution, merely a replacement of one set of plants by another.

And aside from these considerations, every botanist knows the present chaotic state of opinion on the species question, and no ecological principles, especially none so sound as Dr. Nichols'; should be supported on so weak a crutch.

Let me now present my views again in slightly different form. Our problem is to explain the presence of sharply marked areas of vegetation, each characterized by a floristic composition which is essentially uniform throughout its extent, which are generally known as plant associations, or by some other similar term.

My explanation depends on two fundamental assumptions:

First, that flowering plants in general produce a sufficient number of seeds and have sufficient ability for migration to attain a uniform distribution over a wide area, instead of being limited to these definite areal aggregations or "pieces of vegetation," to use Dr. Nichols' expression.

Second, that for each species of plant there is a certain range of environmental requirements, and that a kind of flowering plant can not establish itself except in those habitats where the available environment falls within this range.

In regard to these two basic ideas, Dr. Nichols says "Practically everyone will agree with him," (that is, myself), "in his three fundamental theses," of which these are two. We may pass them as accepted, at least by Dr. Nichols. The first of these two conditions leads toward a complete homogeneity of vegetation, the second toward a segregation of vegetation according to environmental differences.

The presence of areal aggregations of definite floristic composition and vegetational structure in any region must therefore depend on one or the other of two conditions: first, the existence in that region of a group of plants whose environmental demands are so precisely similar that they must be always found together, or second, the existence in that region of a restricted number of environments, which differ from each other so effectively that the vegetation of each is characteristic.

The first of these is negatived by the fact that there is no physiological evidence that plants are segregated by their environmental demands into group which are nearly or quite mutually exclusive. On the contrary, every plant has a considerable range of environmental requirements, from wet to dry, from hot to cold, from dark to light, and all plants together cover such a range of environment that, without another controlling factor, vegetation would show nothing but continuous gradations, and associations in the usual sense would be unknown.

This possible cause is also negatived by the fact that many species which are regularly associated in one region differ greatly in geographic distribution, and each of them extends beyond this region into other environments in which they are nor associated.

The second alternative, that of the presence of a restricted number of environments in any region, is substantiated by the well known fact that different associations are regularly characterized by distinct environments, and by the fact that regions in which environments differ effectively in only one or a few characters, which vary gradually in space, are characterized by corresponding gradual transitions in the vegetation.

As a result it follows that the existence of a uniform vegetation over a measureable extent of space, which feature is solely responsible for the association-concept in its usual form, and the repetition of similar aggregations in the same region, depend on the continuance of a uniform environment over the same extent, and on its repetition in other places, as well as upon uniform opportunities for plant migration.

Now it is well known that it frequently happens that a uniform environment does have a considerable extent and is repeated in other areas. Whereever this happens, a definite plant association develops, and is repeated in neighboring habitats. If this condition was universal, neither my paper nor Dr. Nichols' criticism would have been written. But there are other places it does not occur. There the environment changes so gradually from one place to another that the association-concept in Dr. Nichols' sense is no longer applicable.

The second part of Dr. Nichols' paper deals with the classification of plant associations. As long as we are dealing with a region where the vegetation falls into distinct association, a classification is undoubtedly possible and usually desirable. I have not critically examined Dr. Nichols' last expression on this subject, as presented to you today, but I presume they differ little, if at

all, from his earlier views already published. Those I have studied carefully and I fully agree with them.

The objection to classification, put forward in my own paper, may also need further presentation. We all agree that there may be differences in opinion on the scope of the association. Whether the units be large or small, the possibilities of classification are not affected. The essential feature of the association concept is areal extent, and this implies both size and boundary. And for a fully successful classification a third feature, distinctness, discreteness, non-intergradation, call it what you will, is desirable, as well as a fourth, the existence of areas which can be chosen as typical. As long as our associations have size and boundary, we can proceed to classify. But when the area of uniform environment is too far reduced in size, and our associations reduced to fragments, even the recognition of them hardly appeals to our minds. When the vegetation varies so gradually in space that boundary lines lose their distinctness and the transition zone is lost, we can no longer even select the associations to be classified.

Similar cases of difficulty arise when we deal with matters of vegetational history. When succession is well-marked, we recognize the vegetation of a transition period as such: when it is very slow, the same sort of vegetation may be regarded as an independent association.

Vegetation, then, varying as it does both in time and space, partly through areal variation or chronological change or seasonal or periodic fluctuation in the environment, partly through changes in the flora which by its migration provides the component plants, is a wonderfully unstable thing, and I can only repeat what I have already printed, that a rigid definition of the scope or extent of the association is impossible, and that a logical classification of vegetation into association or larger groups or successional series has not yet been achieved.

Nevertheless the fact still remains that in any vegetation each individual plant casts its influence over its immediate surroundings, modifying the environment to a greater or less degree, aiding thereby in the determinations of its neighbors by environmental selection. There is thus built up a commensalism, to use Warming's old term, of plants which are living together, of plants which are associated, of a plant association under the individualistic concept, while the continuance of the same type of commensalism over an extent of territory leads to a "piece of vegetation," to a plant association in Dr. Nichols' sense.

THE CLIMATIC CHARACTER OF THE LAST INTERGLACIAL PERIOD IN EUROPE[1]

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The great historical successions of floras, which began both in Europe and in North America during the postglacial period, have their starting point in the diluvial glaciation. Its distinct traces are to be seen as well in the old as in the new world. The investigation of those postglacial successions of floras and climates can be based on a solid foundation only as far as it concerns the territiories over which the diluvial glaciation spread. In this case the evidence lies in the fossil floras which permit a strict reproduction of the historical succession. But, when we have once left those territiories and have found ourselves in those parts of the northern hemisphere which have not been subjected to glaciation during the diluvial period (in the so-called diluvial refugiums), then our investigations lose their solid bases, and our ideas of the successions which have taken place there must always remain hypothetical and doubtful.

While treating of the historical problems of the flora in such territories as have not been subjected to the diluvial glaciation, we are fain to use the notion of "relict." Then we often say, indeed too often say "tertiary relict" (Tertiärrelict). This notion is particulary applied to those species of plants which are distinguished by an isolated place in the system, or to those whose repartitions are decidedly disjunctive. When dealing with epiontological considerations of this type, we forget often that the glacial period was not all cold, but had also warm interglacial phases. In consequence of this, we falsely give the name of "diluvial relicts" to glacial plants only, namely, to those species which have an Arctic, Alpine, or Arcto-Alpine character. The "interglacial relicts," or even the possiblity of their existence, are passed by in silence, principally because our knowledge of the length of duration and character of both flora and climate of those phases was till lately scarce.

I believe it is high time for us to revise our views on that subject and to take into account the interglacial periods in all our epiontological examinations respecting the history of both European and American floras. Particularly the last great interglacial period, which belongs in Europe to the time between the two glacial periods, Riss and Würm, should be taken into closer consideration. For, it becomes more and more evident that the interglacial period was

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 17, 1926.

not in the least an unimportant episode in the diluvial time, but that—both as to its duration and as to its specific cycle of development in the flora and climate—it could and even must have left more traces (relicts) in the actual European flora than in the far-off tertiary period (pliocene) of a very different climate.

It has long been known that the flora and the climate of the interglacial periods were characterized by the presence in Central Europe of pretty numerous old types of plants, called "tertiary types" (such as for instance: *Brasenia purpurea*, *Picea omoricoides*, *Rhododendron ponticum*, *Vaccinium priscum* and others). As to the climate, it was—according to an opinion generally acknowledged—warmer and more moist than the actual one (postglacial). The best proof of this is the existence in the European interglacial sediments of such kinds of plants as *Najas flexilis*, *Taxus baccata*, *Fagus silvatica*, *Tilia platyphyllos* and many other—in places situated much farther east and north than are the actual boundary lines of the repartition of these species.

As I do not pretend to give here a detailed picture of these facts, I present on the subjoined map (Fig. 1) the confrontation of the geographical repartitions, both actual and interglacial, of several species of trees. This picture furnishes immediate proof of the great difference between the climatic conditions of the last interglacial period and those of the aluvium.

The above facts—long since known—make us state that the climate of the interglacial periods in Europe was such that it rendered possible the migration of trees belonging to different climatic conditions. For it is clear that the beech (*Fagus silvatica*), or the yew (*Taxus baccata*), which in the last interglacial period have reached as far northeast as the environs of Moscow (Lichwin on the Oka), could not have migrated so far, at the same time that the Pontine Tartary plane-tree has found it possible to extend its vauge to distant Silesia (Ingramsdorf) and the environs of Grodno (Samostrzemlki). One must surmise that the expansion of such heterogeneous floristic elements as those of the Atlantic (*Fagus* and *Taxus*) and those of the Pontine (*Acer tataricum*) did not take place at the same time, although it occurred during the same interglacial period.

For the last 2 years I have devoted much time and labor to obtaining a better knowledge of the flora and climate of the last interglacial period in middle Europe trying to establish approximately the phases of the great historical succession. The starting points of my work were interglacial sediments abounding in plant remains, which have been discovered in Poland during the last few years. As I have already described those floras in another paper[6], I will therefore give here but a short summary of the result of my investigations.

During the last few years there have been discovered in Poland in some localities, distant from one another, rich fossils of the interglacial flora originating from the last interglacial period, corresponding to the time between the Riss

[6] Szafer, W. Über den Charakter der Flora und des Klimas der letzten Interglazialzeit bei Grodno in Polen. Bull. de l'Acad. de Sci. de Cracovie, 1925. Szafer, W. Zur Frage der Vielgestaltigkeit, Herkunft, sowie des Aussterbens von Brasenia purpurea im europäischen Diluvium. Festschrift Carl Schröter, Geobot. Inst. Rübel, Heft 3, 1925.

Fig. 1: COMPARISON OF THE ACTUAL LINES OF REPARTITION OF SOME SPECIES OF PLANTS WITH THOSE OF THE INTERGLACIAL PERIOD.
— LINE OF THE ACTUAL REPARTITION
--- LINE OF THE SUPPOSED INTERGLACIAL REPARTITION.
1. ILEX AQUIFOLIUM
2. TILIA CAUCASICA
3. —"— PLATYPHYLLOS
4. FAGUS SILVATICA
5. ACER TATARICUM
6. TAXUS BACCATA
EUROPE.
Projection Bonne
Meridian of 0 Greenwich
Longitude East 30 from Greenwich
PRINTED IN GREAT BRITAIN BY GEORGE PHILIP & SON LIMITED LONDON

(Gl. 3) and Würm (Gl. 4) glacial periods. A thorough investigation of the fossil flora from 6 localities where it was found (Ludwinów near Kraków,[2] Raków,[3] Sulejow,[4] Szczercowo,[5] Zydowszczyzna[6] and Samostrzelniki[7] near Grodno) has made it possible to retrace a picture of its development and to establish the successive phases of the interglacial climate in Poland. The layers of the interglacial peats, "gytja" and "dy" in the surroundings of Grodno have proved of particular importance because of their rich plant contents, thanks to which almost the whole cycle of evolution of the flora and climate could be reconstructed. This compared with the interglacial flora from other localities in Poland, as well as with fossil floras from the neighboring countries, Germany and Russia, has shown essential concordance.

The facts furnish proof that the last interglacial period in all central Europe bears the same character in its successive phases of vegetation and climate. It must be said, however, that the western countries (Denmark, northern Germany) had, at the interglacial climatic optimum, the warm Atlantic climate and flora, while Poland, situated far inland, was subjected at the time to the continental climate and had in its own thermal optimum, a climate warmer but much drier than the contemporary one of the coastal region. From the moment of the regression of the inland ice of the next to the last glacial period till the time of the southward transgression of the northern glaciers of the last one, there can be discerned in Poland the following, characteristic phases of the flora and climate.

1. *The Arctic phase.* In southern Poland (Ludwinów near Kraków and Krystynopol near Sokal) a woodless "tundra" with a mixed Arctic-Carpathian flora. In northern Poland (Grodno) also a "tundra" with shrubs of *Betula nana*, *B. tortuosa*, *Salix lapponum*, etc.—Arctic climate.

2. *The sub-Arctic phase.* In southern Poland (Ludwinów) there appear upon the tundra the first forest composed of *Pinus cembra*, *Larix* sp., *Pinus silvestris*, etc. In northern Poland (Grodno) there appears *Pinus silvestris*.—Subarctic climate.

3. *The boreal phase.* Near Grodno there appear pine and oak forests. First a cooler, later a warmer continental climate.

[2] Zmuda, A. J. Die diluviale Flora des Krakauer Diluviums. Bull. de l'Acad. de Sci. de Cracovie 1914. Lilpop, J. and Szafer, W. Contribution to the knowledge of the flora and the climate of the Polish diluvium. Spr. Panstw. Inst. Geol. Vol. 1, sh. 4–6, Warsaw, 1922. (In Polish.)

[3] Koslowska, A. The interglacial flora in Rakow. Acta. Soc. Bot. Poloniae, Vol. 1, Warsaw, 1923. (In Polish.)

[4] Lilpop J. and Passendorfer, E. Diluvian Flora in Sulejów. Spr. Panstw. Inst. Geol. 1925. (In Polish.)

[5] Premik, J. Geological research-work on the Widawka. Spr. Panstw. Inst. Geol. 1925. (In Polish.)

[6] Papers relating to the fossil flora of Zydowszczyzna are as follows: Szafer, W. Über den Charakter der Flora und des Klimas der letzten Interglazialzeit bei Grodno in Polen. Bull. de l'Acad. de Sci. de Cracovie, 1925. Szafer, W. Zur Frage der Vielgestaltigkeit, Herkunft, sowie des Aussterbens von Brasenia purpurea im europäischen Diluvium. Festschrift Carl Schröter, Geobot. Inst. Rübel, Heft 3, 1925.

[7] Szafer, W. Eine Dryas-Flora bei Krystynopol in Galizien. Bull. de l'Acad. de Sci. de Cracovie 1910.

4. *The I sub-Atlantic phase.* There appear forests with *Abies alba, Taxus baccata, Carpinus Betulus,* etc. In the waters: *Trapa natans, Najas major, N. flexilis,* etc.—Suboceanic climate approaching the present one.

5. *The pontish-meridional phase.* In all Poland the coniferous trees recede and instead the following begin to dominate: *Tilia platyphyllos, Acer tataricum, Carpinus Betulus,* etc. In the waters: *Trapa natans, T. muzanensis, Brasenia Schröteri, B. Nehringii,* etc.—Thermal climate optimum, with a meridional-pontish character.

6. *The II sub-Atlantic phase.* (Grodno, Ludwinów, Raków [?], Szczerców.) Beech and fir forests (*Fagus silvatica, Albies alba* with *Carpinus Betulus, Tsuga* sp., etc. In the waters there are still persisting *Brasenia Schroteri*, but *Brasenia Nehringii* is dying out.—Sub-oceanic climate.

7. *The presub-Arctic phase.* (Sulejów, Szczerców, Grodno.) Pine forests (*Pinus silvestris*) and northward also spruces (*Picea excelsa*). The thermophile water-flora dying out or regressing southwards.) Cold climate.

8. *The Arctic phase.* (Szczerców.— Gradual disappearance of the forests and reappearance of the "tundras" with *Betula nana.* There glide down into Poland the tongues of the northern glaciers.—Sub-Arctic climate changing into an Arctic one.

The successive phase of the flora and climate during the last interglacial period in Poland are strikingly like those which have been described for the postglacial period (Sernander), with one difference, however, namely, that the thermal optimum rises higher in this last interglacial period than in the postglacial one.

The above schema of 8 phases of the development of the flora and climate of the last interglacial period in Poland, cannot be considered as absolutely certain. Much less can it be applied to all central Europe. Nevertheless, it seems probable that its meaning is to a certain extent general, though of course it cannot be adapted to the interglacial period of western Europe, which has not had the meridional-Pontish phase. It has also differed from the Russian interglacial period, which was generally cooler and more moist. These differences of facies, briefly mentioned here, must be elaborated by further, more detailed, stratigraphic investigations of the interglacial deposits in different parts of Europe.

The problem of the migration of diluvial plants in the light of the thoroughly confirmed cycle of the succession of the flora and climate in the last interglacial—appears quite different from what it had been generally considered up to this time. For, it is clearly established that the long duration as well as the specific climatic changes in this period have permitted the migration of these plants which belong to very different geographical elements, and which require quite different climatic conditions. In the periods 1, 2, 7 and 8, the Arctic, sub-Arctic, and Alpine elements could effect distant migrations. In the third period—the dry one— and in the fifth, or continental one, the steppic and substeppic plants could easily extend greatly their vauges. The fourth and sixth periods were the times of the expansion of the atlantic and the subatlantic elements.

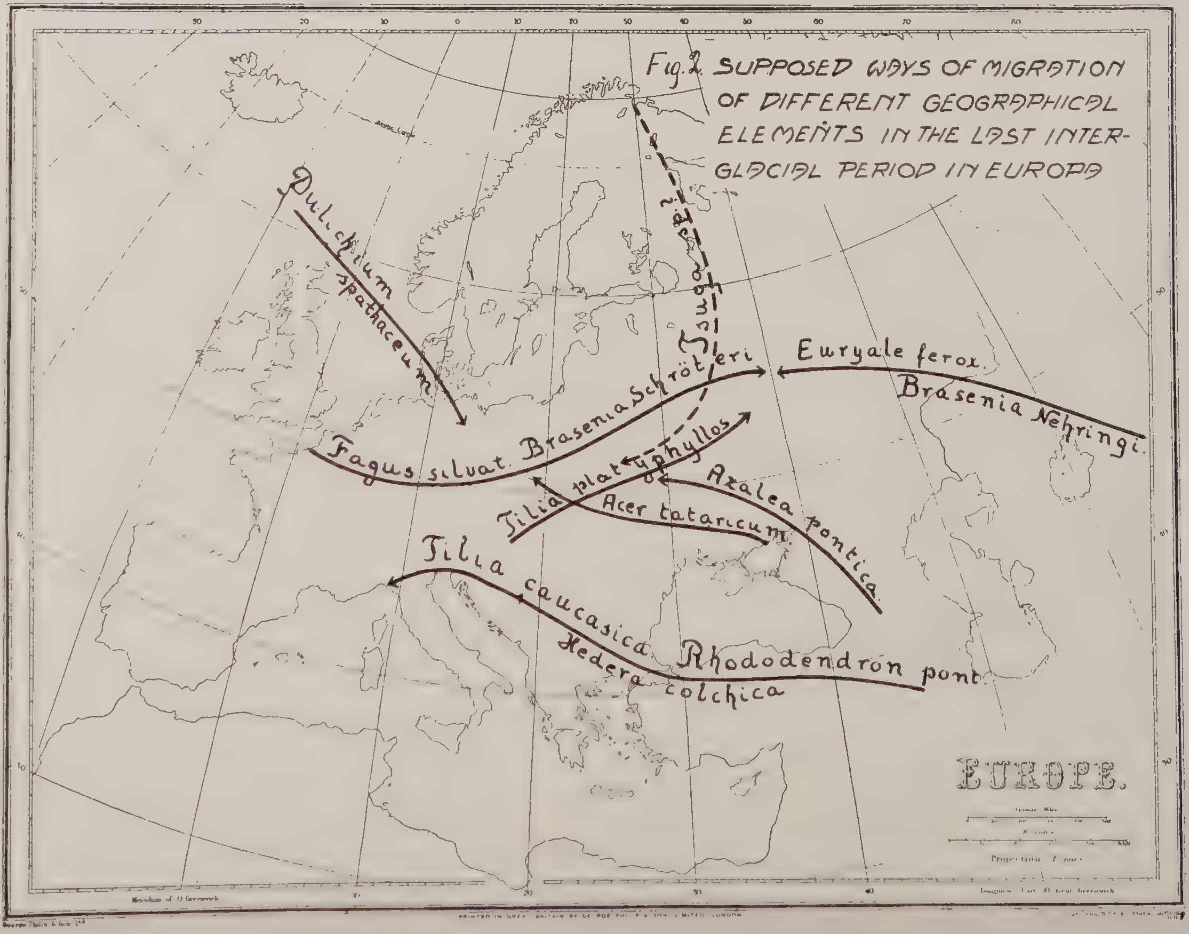

Fig. 2. SUPPOSED WAYS OF MIGRATION OF DIFFERENT GEOGRAPHICAL ELEMENTS IN THE LAST INTERGLACIAL PERIOD IN EUROPA

That such migrations of various elements were effected on a large scale in the interglacial period is undoubtedly proved by their fossil remains found often at a distance of hundreds of kilometers from the actual lines of repartition of the particular species.

A proof of this statement is offered in the subjoined map (Fig. 1). We see there some lines of the actual and interglacial repartitions of plants (in accordance with out present knowledge) belonging to different geographical elements. The great extension of repartition of those species during the interglacial period can be accounted for only by the different climatic phases of this period.

I do not aim at reproducing here an approximate picture of the paths of the plant migrations in Europe in the last interglacial period. I have, however, sketched it on the accompanying map (Fig. 2), basing it on a small fragment of the interglacial flora of Europe. Further investigations alone will permit us to develop our knowledge also in that respect. At any rate, it is certain that in the interglacial periods the flora of central Europe was under the influence of the waves of plant migrations progressing from far distant refugiums situated in the far East (*Euryale*), in the West (*Dulichium*) and in the South. Therefore, a great many of the species nowadays called "tertiary relicts" might have migrated to central Europe from even very distant territories in the interglacial periods (*Rhododendron ponticum* from Asia Minor).

These facts demand, I believe, an earnest revision of our present considerations on the question of the "historical relicts" in general. For our veiws of the distribution of "tertiary relicts" have, been on the one hand, certainly very problematical until the present time while, on the other hand, the part played by plants in the interglacial age was strikingly undervalued.

BEITRÄGE ZUR KENNTNIS DER PROBLEME DER INSULAR FLOREN UND VEGETATIONEN[1]

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Ich nehme mir vor ihre Aufmerksamkeit auf die Vegetation und Flora einer kleinen Insel zu lenken, die im Schwarzen Meere, unweit der rumänischen Küste, in der trockensten Steppenzone Osteuropas liegt, und uns manche interessante Aufschlüsse über die Konstitution der Vegetation und das Wandeln der Floren in den Steppen geben kann.

Wir kannten ja bisher nur die Entwicklung der Flora mancher tropischen Inseln aus den Arbeiten von Treub (1888, 1897, 1902-mit Penzig), Ernst (1907, 1909, etc.) und die Konstitution der Vegetation nordischer Inseln der Laubwaldzone, besonders aus den trefflichen Studien Palmgrens (1922, 1925).

Die Schlangen-Insel liegt 45 km. weit von der Sulina-Mündung des Donau-Deltas im Schwarzen Meere. Sie ist kaum 17 ha. gross und erhebt sich 5–17 Meter über das Meeresniveau. Diese kleine Insel ist aus permischen kieselreichen Sandsteinen, Conglomeraten, und Quarziten aufgebaut, und ragt mit steilen Wänden aus dem Meere heraus. Nur an der Nordseite gleiten erdige Schichten bis an das Wasser herunter. Hier kommen Terrarossa-artige Erden zwischen den Felszungen hervor. Das Plateau selbst ist halbwegs mit humusreicher Erde bedeckt, die hie und da bis $1\frac{1}{2}$ m. Dicke besitzt und einer Loessschicht aufsitzt. In den höheren Punkten ist die Erdschichte sehr dünn und beinahe aus rohem oder halbzersetztem Guano zusammengesetzt. Die Insel ist nämlich in ihren felsigen Partien der Nistplatz von Hunderten von Silbermöven. Zur Zeit des Vogelzuges lassen sich auch oft Hunderte der verschiedenen nordeuropäischen Vögel herab. Von Säugetieren sind nur eine kleine Schar Schafe, die zeitweise in die Ökonomie des Pflanzenlebens eingreifen und den Boden mit organischen Stoffen bereichern.

Der pH-Gehalt des humusreichen Bodens ist über 6.5, des steinigen rötlichen Ufers 4.85–3.7, des Loesses aus der unteren Erdschichte 4.5.

Die Schlangen-Insel ist erst seit der letzten postdiluvialen Transgression endgültig trocken. Das Klima ist jetzt ein äusserst trockenes Grasflurklima mit Niederschlägen unter 366 mm. pro Jahr, auch diese auf den Winter und das Frühjahr verteilt, mit regenlosen, trockenen Sommern, mit heiterem Himmel und grosser Lufttrockenheit. In den Wintermonaten herrschen heftige Winde. Die wellige Beschaffenheit der Oberfläche verursacht einen sehr raschen Abfluss des Regenwassers.

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 19, 1926.

Das Pflanzenleben wurde wissenschaftlich im Jahre 1841 von Nordmann, 1912 von Miss Pallis und Enculescu, 1925 von Savulescu, 1923, 1924, und 1926 von Borza untersucht.

Die Insel ist in ihrer Gesamtheit mit einer Grasvegetation bekleidet, am Ufer mit einer reichen Meeresalgen-Vegetation geschmückt. Von Phanerogamen, die uns hier allein beschäftigen, sind bisher auf der Insel insgesamt 60 Arten bekannt. Von diesen sind beinahe 30 Prozent Anthropophyten, und nur 70 Prozent von der Tätigkeit des Menschen mehr oder minder unbeeinflusst.

Nach ihrer aktuellen geographischen Verbreitung sind weitverbreitete eurasiatische und im allgemeinen boreale Arten, also Ubiquisten, einige sogar Kosmopoliten, 42 (50 Prozent), mit pontischer (pontico-sarmatischer und balkanischer) Verbreitung 8 Arten, mediterran bis pontisch-mediterran 10 Arten, von welchen *Trifolium subterraneum*, *Taraxacum megalorrhizon*, *Trifolium intermedium* und *Ornithogalum refractum* die bezeichnendsten sind.

Der Florencharakter der Insel ist also pontisch-eu-mediterran und zeigt *die bemerkenswerte Resistenz der alten eu-Mediterran-Elemente*, die ich auch paleopontisch nennen möchte, gegen die sich immer mehr ausbreitende Anthropophytenflora, und der echten pontischen Flora, die ich neopontisch bezeichnen möchte.

Auffallend klein ist die Artenzahl dieser Inselflora, wenn man bedenkt, dass der permanente Grundstock der Phanerogamen kaum 40–50 ist, die übrigen 15 Arten nur vorübergehend auftreten und dann wieder verschwinden. Es fehlen da die meisten an der nächsten rumänischen und russischen Küste vorkommenden echten Steppenelemente: *Stipa*-Arten, *Iris*-Arten, Centaureen und Artemisien, *Ceratocarpus*, *Carduus*-Arten, etc., obwohl nicht geeignete Standorte fehlen.

Das bezeugt also, dass sich die Steppenflora sprungweise schwerlich verbreiten kann, dass sie vielmehr nur schrittweise in compakter Arten-zahl vorrückt.

Es fehlen alle Endemiten der reichen, alten Dobrudscha-Flora, die eine praediluviale Reliktenflora ist. Diese Tatsache bestätigt auch den geologischen Befund über die Jugend der Insel.

Von bemerkenswerten Formen, die hier wahrscheinlich neue ökologische Adaptation zeigen, sogenannte Kampfformen, nenne ich *Matricaria chamomilla* for. *debilis*, dann *Trifolium intermedium* for. *hirsutior* und *Vulpia myuros* for. *breviglumis*.

Dass das kleine Areal der Insel auch ein Hauptgrund der vorhandenen kleinen Artenzahl sei, kann mit genügendem Grunde behauptet werden, ohne diesem Erscheinen in sich, ohne genügende Rücksicht auf die physikalischen Bedingungen, eine zu grosse theoretische Bedeutung beizumessen, wie es Palmgren für Aland feststellen konnte. Im Vergleich mit Inseln derselben Grösse, wie Aland, sind hier viel weniger Arten zu finden. Was die Migration und Besiedelungsmöglichkeiten der hier beobachteten Blütenpflanzen anbetrifft, kann ich meine Beobachtungen und Experimente im folgenden zusammenfassen:

Die Meereswellen, besonders der N-S Küstenstrom, bringen ganz sicher vegetabilen Detritus auf die Insel mit sich. Ich fand da im April 1924 ansehnliche Stämme von Weiden, die hier allerdings nicht weiterleben und Wurzel fassen konnten, im botanischen Garten von Cluj aber, in die Erde gesetzt, sich weiterentwickelten. Die massenhaft auf die Insel geschleuderten Trapa natans-Samen waren auch leer. Es fanden sich da auch noch viele lebende *Phragmites communis*-Zweige, zusammen mit abgestorbenen "Plaw" -Stücken, mit Fragmenten der aus den Arbeiten von Miss Pallis, Enculescu, und Antipa bekannten schwimmenden Pflanzendecke des Donau Deltas. Ganz gewiss kommen auf diesem Wasserwege alle möglichen Sämereien auf die Insel, von welchen aber wegen der ungünstigen physikalischen Verhältnisse nur wenige zur Keimung gelangen.

Mit den gewöhnlichsten dieser 21 Arten dieser Inselflora stellte ich Versuche an, um ihr Verhalten im Meereswasser festzustellen. Ich legte in Wasser von der Zusammensetzung des Schwarzen Meer-Wassers die Samen in gesonderte Schalen und fand, dass nach siebentägigem Liegen im Wasser alle in Sand ausgesäten Samen keimten, und zwar in 60–90 Prozent. Nur *Rumex acetosa* und *R. crispus* keimten kaum in 20 Prozent. *R. acetosella* versagte ganz, ebenso *Calamagrostis epigeios*. Nach dreissigtägigem Liegen in Wasser keimten noch alle übrigen Spezies in 10–70 Prozent der Fälle. Erst nach 45 Tagen scheinen ihre Keimfähigkeit gänzlich verloren zu haben: *Setaria viridis*, *Bromus longipilus*, *Erigeron canadensis*, *Rumex crispus*, *Portulaca oleracea*, *Malva neglecta*. Nach 60 Tagen keimten nicht mehr: *Matricaria chamomilla*, *Lepidium ruderale*, während *Agropyron repens* (in 30 Prozent), *Bromus hordaceus*, *Vulpia myuros*, *Taraxacum officinale* (10%), *Lactuca scariola* (20 Prozent), *Matricaria inodora* (30 Prozent), *Rumex acetosa* (30 Prozent), *Stellaria media* (70 Prozent), *Geranium pusillum* (30 Prozent), *Amaranthus retroflexus* (40 Prozent), und *Anthriscus cerefolium* (20 Prozent) in ausgewaschenen Sand gelegt, noch immer schön keimten.

Der grösste Teil der auf der Insel vorhandenen Pflanzen kann also durch das Wasser hingebracht sein, schwimmend, bzw. auf herumschwimmenden grösseren Objekten.

Die Rolle der durchziehenden Vögel im Einführen der Pflanzen kann nicht sehr hoch eingeschätzt werden. Es fehlen auf der Insel gänzlich passende Standorte für Sumpfpflanzen, die von den Wasservögeln gebracht werden. Hingegen könnten endozoochor manche Samen auf die Insel gekommen sein. Experimental kann man der Sache schwer nachgehen.

Der Mensch hat seit dem griechischen Altertume diese Insel gekannt, hier Tempel gebaut und Opfertiere eingeführt. In den vierziger Jahren des vergangenen Jahrhunderts waren dort noch Roggenkulturen. Jetzt wohnen dort permanent 4–5 Wächter des Leuchtturmes der europäischen Donau Kommission. Diese Kulturtätigkeit musste auch zur Einführung zahlreicher Sämereien führen. In den letzten Jahren stellte ich das ganz sporadische Auftreten einiger Unkräuter fest die dort früher nie gesehen worden waren: *Sinapis arvensis*, *Brassica rapa* var. *campestris*, *Polygonum aviculare*, im Hofe des Leuchtturmes, wo verschiedene Bauten aufgeführt worden waren. Ob sich diese neuen Bürger der Schlangen-

Insel-Flora wohl da halten können? Die üblichen Roggen Unkräuter sind mit diesen Kulturen ganz verschwunden.

Der Wind bringt sicher auch viele und verschiedene Samen auf die Insel über die 42–100 km. breite Wasserbarriere. Auffallend gering ist jedoch die Zahl der sicher anemochoren Kompositen, die den ökologischen Bedingungen der Insel gewachsen sind; es sind dies Kompositen, die zufällig auf die Insel kamen und durch die strenge Auslese der physikalischen und biotischen Faktoren zugelassen wurden. Es ist also einleuchtend, dass der Anteil des Windes an dem Zusammenbringen der Inselfloren kleiner ist als der der übrigen Transportmittel, wie das übrigens auch Ridley (1923) vermutete.

Morphologie und Ökologie der Pflanzendecke

Das ganze Plateau der Schlangen-Insel ist mit einer Grassteppenvegetation dicht bekleidet, dessen dominierende Hauptkomponenten im allgemeinen *Bromus longipilus*, *B. hordaceus*, und *Hordeum leporinum* sind. Diese drei vorherrschenden Elemente mengen sich in der weiten Steppe in verschiedener Weise und desto mehr wechseln die anderen Komponenten. Man kann also nicht nur verschiedene Fazies, sondern auch einige ziemlich gut charakterisierte Subassoziationen unterscheiden. Im folgenden gebe ich nur die Abundanz in weiterem Sinne, die Frequenz und Soziabilität der Elemente an. Bei der geringen Ausdehnung der Insel und bei dem vorherrschenden Anteil der Ubiquisten und Ruderalelemente kann man von Gesellschaftstreue und Konstanz kaum sprechen.

(A) Das Brometum hordacei ist am reichsten im Zentrum der Insel mit dem dicken Humusboden, dessen Wasserstoffionen-Konzentration pH = 6.57 ist.

Bromus hordaceus 552, *B. longipilus* 243, *Hordeum leporinum* 554, *Trifolium subterraneum* 245, *Ornithogalum refractum* 212, *Cerastium pallens* 131, *Holosteum umbellatum* 131, *T. strictum* 113 *Rumex acetosella* 114, *Vulpia myuros* 124, *Valerianella costata* (present) *Taraxacum megalorrhizon* present.

Diese Subassoziation ist phytosoziologisch die reifste, eine relative Klimax-Assoziation, mit einer ziemlich gleichmässigen Dispersion der Komponenten und mit einer vorzüglichen komplementären Ausnützung des Wurzelortes und Luftraumes. Allerdings kann man sie aber nicht als floristich vollkommen saturiert bezeichnen. Im Winter und Vorfrühling zeigt dies Brometum ein frisches grünes Kleid, im Frühling schmückt es sich mit den weissen Sternchen des *Ornithogalum* und den pygmäischen Ephemeren *Cerastium* und *Holosteum*. Im Juni ist schon alles trocken und bräunlich verfärbt, nur in den Depressionen herrscht noch das grünlichere Kleid des *Hordeum*.

(B) Auf steinigen, weniger humusreichen Stellen ist das Brometum ärmer, dort wo pH = 6.1 ist. Es tritt als bezeichnende Differentialart *Matricaria chamomilla* auf.

Bromus longipilus 542, *Matricaria chamomilla* 452, *Holosteum umbellatum* 341, *Cerastium pallens* 341, *Malva silvestris var. eriocarpa* 111, *Rumex acetosa* 111, *Geranium pusillum* 111, *Vulpia myuros* 211.

(C) An vier ausgedehnten Plätzen mit vorragenden kieseligen Felsen nisten jahraus-jahrein Hunderte von Silbermöven. Der Boden ist mit viel Guano

bedeckt. Das Brometum verändert sich gänzlich, die Vegetation hat hier ein kolonien- oder mosaikartiges Aussehen, dessen vollkommenes Ausreifen durch die permanente Aktion der Vögel verhindert wird.

Bromus longipilus 122, *Matricaria chamomilla* 521, *Atriplex tataricum* 554, *Rumex acetosella* 12, *Hordeum leoporinum* 114, *Holosteum umbellatum* 154, *Calamagrostis arundinacea* 215 (lokal).

Diese drei Subassoziationen repräsentieren auch die drei Etappen der Entstehung des Bodens und der Sukzessionen der Vegetation, Sukzessionen, die allerdings lange dauern müssen, mit der Bodenentwicklung parallel verlaufen, und in zoogenen Hemmungsstadien sich lange erhalten können.

Die steil abfallenden Nord-, Ost- und Süd Küsten tragen an den erdigen Stellen eine üppige Vegetation, die aber sehr lose ist und Kolonisten-Charakter besitzt, morphologisch und phänologisch nicht reif, nicht saturiert ist. Der Boden zeigt auch eine grosse Azidität, die zwischen pH = 3.7 und 4.85 schwankt. Diese Vegetation ist kolonienartig zusammengesetzt aus Beständen von *Matricaria chamomilla*, die am Nordabhange auch einen reinen, ausgedehnten Bestand bildet; andererorts finden sich Bestände von *Atriplex tataricum*, *Agropyron dumetorum*, *Rumex crispus*, *Hordeum leporinum*, *Calamagrostis arundinacea*, *Malva silvestris* var. *eriocarpa*, und vereinzelt *Taraxacum megalorrhizon*. Von allen scheint *Hordeum leporinum* die Tendenz zu haben, von den meisten kleinen Terrassen Besitz zu ergreifen.

Eine geschlossene, reife Gesellschaft entsteht hier doch nicht, weil der Boden leicht abrutscht, die Aktion des Frostes zerstörend wirkt und auch die Wellen bei heftigen Stürmen Boden und Vegetation arg angreifen.

Bemerkenswert ist die hartnäckige Widerstandskraft des *Taraxacum megalorrhizon* und des *Rumex crispus* mit sehr alten Wurzelstöcken und Pfahlwurzeln.

Das biologische Bild der Vegetation der Insel insgesamt genommen ist folgendes: Therophyten 90 Prozent, Hemicryptophyten 4 Prozent, Geophyten 6 Prozent. Diese Tatsache erklärt auch die Saison-Aspekte und auch die Unbeständigkeit der Pflanzendecke auf dieser Insel sowie überhaupt in den Steppen. Die überwältigende Mehrzahl der einjährigen Pflanzen kann der Vegetation nicht jährlich denselben Rhytmus sichern. Es entsteht vielmehr eine Konkurrenz, in welcher die früher keimenden bald die Oberhand gewinnen.

An zwei kleinen, mit primitiven Steinmauern umgebenen Plätzen, die vor dem Wind etwas geschützt sind, werden auch Küchengewächse angebaut, wie Kohl, Kartoffeln, Petersilie, Zwiebeln, Tomaten, Eieräpfel, Melonen und Mais. Reich ist hier die Unkrautvegetation, besonders dicht an den Mauern: *Geranium rotundifolium*, *Malva silvestris* var. *eriocarpa*, *Anthriscus scandix*, *Agropyron repens*, *Capsella bursa pastoris* riesiger Form, *Galium aparine*, besonders viel *Urtica urens*, *Chenopodium album*, *Stellaria media*, *Rumex crispus*, *Hordeum* und die zwei *Bromus*-Arten der Insel, die hier zwischen den vielen Mesophyten auch eine üppigere Mesophytengras Konsistenz haben.

Bemerkenswert ist, dass in einigen tiefen Klüften, die immer im Schatten liegen und luftfeuchter sind, sich Stellaria und Galium reichlich vorfinden. Das

bezeugt, dass nicht der Regenfaktor im Minimum, sondern die grosse Verdunstung und Lufttrockenheit auf dem Plateau die Mesophyten ausschliesst.

Die zum Vorschein kommenden Felsen sind mit dichtem Flechtenteppich bedeckt, auf den ich hier nicht näher eingehe.

Auf den untersten Bänken des Ufers, die vom Wasser reichlich bespritzt werden, finden sich üppige und besonders grossblütige und wohlriechende *Matricaria chamomilla*-Exemplare. An schmalen Schlupfwinkeln ist *Spergularia marginata* zu finden. An anderen Orten ist alles mit üppigen Atriplexen besetzt. Diese notorischen Halophyten vertragen also am besten die Salze des Meeres. Vereinzelt kommen aber auch von den Gramineen *Hordeum*, und auch hohe Stöcke von *Rumex acetosella* vor.

Die entsprechend den topographischen Verhältnissen überhaupt mögliche zonale Verteilung der Gewächse gemäss dem Salzfaktor ist hier also sehr evident.

Experimentelle Einzelbeobachtungen über die Vitalität und Konkurrenztätigkeit der Komponenten der dominierenden Grasflur sind noch nicht abgeschlossen, doch ist es sicher, dass in diesen Steppen mit vielen einjährigen Pflanzen und jährlich wechselnder quantitativer Zusammensetzung das rasche Wachstum die wichtigste Eigenschaft ist, die im Wettbewerbe um den Platz in der dichten Pflanzengesellschaft ausschlaggebend ist. Langsam wachsende Keimlinge, die nicht an den hastigen Rhytmus des Steppenklimas und Pflanzenlebens gewöhnt sind, werden von den rasch aufschiessenden Gräsern rücksichtslos unterdrückt. Hiedurch wird auch die phytobiotische Auslese der neuen Ankömmlinge durchgeführt. Auf der Schlangen-Insel keimen die Therophyten meist im Spätherbst. Oktober und November ist also die Periode der Auslese in den Steppen. Die im Frühjahre keimenden Chenopodiaceen können ihre Keime nur noch auf graslosen Plätzen entfalten, also auf an Nitraten und Chloraten reichen grasfeindlichen Orten. Ebenso muss es sich mit vielen Pflanzen, besonders Unkräutern verhalten, die während der Winterstürme von den Wellen angeschwemmt werden und die von den ungünstigen Boden- und Klimaverhältnissen noch zugelassen würden, aber der biotischen Konkurrenz zum Opfer fallen.

Endlich machte ich Beobachtungen über die Rolle des Weidens der Schafe und des Nistens der Vögel auf dieser Insel, die wegen ihrer Isolierung als typisches Experimentierfeld betrachtet werden kann.

Bemerkenswert ist in erster Linie, dass die klimatischen Verhältnisse das Gedeihen des Grases während des ganzen Winters gestatten, so dass die 10–20 Köpfe zählende Schafherde im Freien weiden kann. Es sind zu dieser Zeit die frischen Bromus-Halme, die als Nahrung dienen. Dabei werden alle übrigen Unkrautblätter abgefressen. Die winzigen *Cerastium* und *Holosteum* Blattrosetten aber entgehen der Dekapitation.

Vom Frühsommer angefangen werden die immer magerer werdenden und bald ganz austrocknenden Grashalme mit ihren stechenden Früchten nicht mehr angerührt; es sind vielmehr die übrigen Kräuter, die den Schafen zum Opfer fallen. *Rumex acetosella*, *Matricaria*, *Sonchus* und alles Übrige, was nicht sticht und übel riecht, wird abgeweidet. Es fallen so ganz sicher viele, in wenigen

Individuen vorhandenen, Kräuter den Weidetieren zum Opfer. Von den in 1912 vorhandenen Kräutern sind nach meinen dreijährigen Beobachtungen in 1923, 1924, und 1926—also nach dem intensiven Abweiden in den Jahren 1920-1926—gänzlich verschwunden: *Portulaca oleracea*, *Epilobium tetragonum*, *Lactuca scariola*, *Erigeron canadensis*, *Setaria viridis* und *S. glauca*, *Filago arvensis*, *Solanum nigrum* und *S. miniatum*, *Amaranthus retroflexus*, wenn man von einigen unsicheren früheren Angaben absieht. Durch das Abweiden wird also die Steppenflora in solchen gutumgrenzten kleinen Gebieten immer ärmer und einförmiger. Paczosky's und Sukatschew's Beobachtungen und Feststellungen betreffend eine Bereicherung der Steppenflora in weiten Steppengebieten, bewähren sich hier nicht, sind also nicht allgemeingültig.

Wo Schafe länger stationieren oder abends einkehren, wird der Boden durch ihre Exkremente und deren Nitratgehalt pflanzenfeindlich und trägt dann kaum noch Rumex acetosella.

Die hier nistenden Vögel greifen in den Wandel der Vegetation nicht nur chemisch ein, sondern sie verursachen durch mechanische Verletzungen unter den höheren Gramineen eine Verheerung. Das hat aber kaum einen bemerkenswerten Einfluss auf die Zusammensetzung der Pflanzendecke.

Das Studium der Vegetation und Flora der kleinen Schlangen-Insel im östlichen europäischen Steppengebiete führte also zu folgenden Ergebnissen, zur Erkennung oder Bestätigung folgender Tatsachen und Gesetzmässigkeiten von allgemein biologischem Interesse:

Eine breite Wasserbarriere ist in der Steppenzone Osteuropas ein viel bedeutenderes Verbreitungshindernis als in den Tropen oder in den gemässigten Zonen mit nasserem Klima und sie verlangt eine lange Kompensationstätigkeit des Faktors "Zeit."

Die Steppenvegetation kann in kompakter Artenzahl nur schrittweise vordringen, nicht sprungweise.

Die Flächenausdehnung hat für die Artenzahl eine gesetzmässige Bedeutung nur rücksichtlich der physikalischen Faktoren des Standortes.

Das wichtigste Verbreitungsagens ist für die Inselfloren in diesem Falle das Wasser. Wind, Vögel und Menschen spielen eine untergeordnete Rolle.

Die Unbeständigkeit und Unausgeglichenheit in der floristischen Zusammensetzung und in den Mengenverhältnissen des Vegetationsrasens ist der überwältigenden Zahl der Therophyten zuzuschreiben, die eine alljährlich sich erneuernde Konkurrenz möglich machen, deren kritische Ausleseperiode der Spätherbst (und Vorfrühling) ist.

Rasche Wachstumsfähigkeit ist der wichtigste Faktor der Vitalität der Arten, der den Sieg im phytobiotischen Ringen sichert.

Die phytosoziologische Reife der Steppenpflanzen-Gesellschaften hält gleichen Schritt mit dem zunehmenden pH-Gehalt des Bodens.

Die Verdunstung im Maximum—noch mehr als der Wasserfaktor im Allgemeinen im Minimum—ist für die Zulassung der durch Zufall angekommenen

und für die Verteilung der auf diese "Konstellation der ökologischen Faktoren gestimmten" Gewächse von überwältigender Bedeutung und Evidenz.

Auch in den ausschliesslich aus einjährigen Gewächsen zusammengesetzten Grassteppen ist die letzte phytosoziologische Einheit nicht eine Synusie oder Saisongruppe, sondern der aus mehreren Lebenslaufgruppen, Synusien und Schichten zusammengesetzte Pflanzenkomplex einer topographischen Einheit, die wenigstens ein Jahr lang dauernde Assoziation, dessen sämtliche Komponenten—wenigstens in Samenform—wirklich zusammen leben.

Das permanente Weiden führt in kleineren Steppengebieten zu einer Verarmung der Flora.

THE WARM POSTGLACIAL PERIOD AND THE POSTGLACIAL CLIMATIC DETERIORATION OF NORTHERN EUROPE[1]

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The discovery of fossil oak (*Quercus Robur*) and hazel (*Corylus avellana*) in areas where these plants are rare or altogether missing nowadays, and of isolated localities for these species outside their present range, long ago brought men like Elias Fries, Areschoug, and others to the conclusion that the climate of Northern Europe had been milder than it is know. For a long time it was believed that a climatic optimum was reached even before the maximum ofthe Litorina depression and that later a slow but steady fall of temperature follo wed, lasting until now.

Against this I have shown that there was no continuous fall of temperature, but that a well-defined warm post-glacial period (Sernander 1910) was brought to an end long ago by a climatic deterioration. Lennart von Post, at present the leader of peat-bog investigaticns in Sweden, recently altered the name into "the warm post-Arctic pericd," but the term "pcst-glacial"—in oppositionto "inter-glacial"—seems to me to be more expressive. The warm post-glacial period is often confused with the post-glacial climatic optimum, which, however, is only a part of the former not yet well defined.

This warm period includes the last part of the Ancylus-time and the whole of the Litorina-time, that is, the boreal, atlantic, and sub-boreal periods. Archeologically most of the Stone Age and all of the Bronze Age are included in this climatic period.

The deterioration of the climate seems to have set in like a catastrophe, even if certain phenomena that occurred towards the end of the sub-boreal time announced what was going to happen. This catastrophe lasted a few centuries. It forms the limit between several important epochs, between the sub-boreal and the sub-Atlantic period, between the Litorina and the Limnaea time, also roughly between the Bronze and Iron Ages of the North.

The climatic deterioration is distinctly noticed in quaternary layers of different kinds. In the peat bogs the sub-boreal drying-up horizon is brought to a sudden stop, and above follows a peat of a pronounced hygrophilous origin. This sub-Atlantic-sub-boreal contact line has been observed, for instance, by Weber and was described by him from peat bogs in northwestern Germany as the "Grenzhorizont" (limit horizon).

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 19, 1926.

The sub-boreal marine layers around the Baltic are not very thick, but they are rich in halophilous shells and diatoms, showing that the supply of fresh-water rich in sediment was very scanty during that period, while the sub-Atlantic time brought with it the water of the Limnaea Sea with its brackish fauna and flora (Sernander 1911).

For the most severe part of the climatic deterioration I have chosen the name "fimbulwinter" (the great winter) of the old Edda tales. According to this view it had a fatal influence upon the northern tribes of that time, and their culture. It not only meant a decrease of temperature during the period of growth but probably also shortened the growth period.

A classical example is afforded by the former distribution of the hazel in northern Sweden, north of its present limit. According to Gunnar Andersson this extension of its present area comprises over 84,000 square kilometers. As I have shown sub-fossil nuts are found even in the uppermost parts of the sub-boreal strata, but never above them.

Still better, perhaps, this influence is illustrated by a comparison between the post-glacial and present-day distribution of the water-nut (*Trapa natans*). While *Trapa* is often found in sub-boreal layers, it is quite absent in sub-Atlantic time except as a relict in some few localities.

Nevertheless, the devastating effect upon the culture of the North is not so much due to the decrease of the average temperature as to the variation and the insecurity of the climatological constants, that have a decisive influence on agriculture.

Climatic changes and a large amount of rain, as compared with what we get now, were other important features of the sub-Atlantic time.

Northern species migrated from northern Sweden into certain plant communities of the middle part of that country (sub-Atlantic glacial relicts).

The fimbulwinter caused considerable migration among the tribes as well as changes in their life conditions. As a result of the re-expansion of the Scandinavian culture after the climate had reached its present character, at the end of the fimbulwinter, cattle breeding became the principal branch of farming on account of the formation of moist meadows. I have tried to show (Sernander 1925) that the settlements ending in "sta" (of the type Nasta, Bista, Ånsta, etc.) round the lakes Mälaren and Hjälmaren are confined to that type of a country and to the centuries immediately after the birth of Christ. In the year 1912 I made the following remarks about the chronology of the climatic changes (Sernander 1912, p. 145): "Thanks to the collaboration with Swedish archaeologists I have been able to point out that finds from the 2 first periods of the Older Iron Age of Montelius (500–150 B.C.) are characterized by a striking poverty, not only in number but also in quality, in contrast to the overflowing richness during the next 2, the last periods of the Bronze Age (1050–650 B.C.), and that they are concentrated in climatically favored spots in Sweden. Period VI of the Bronze Age (650–500 B.C.) takes an intermediate position."

Gams and Nordhagen have been able to prove not only that my warm period corresponds to the "période xerothermique" of Briquet for the continent of

Europe, but also that a shorter period with an insular climate, that is, the equivalence of the Atlantic period, is included in it. They fix the beginning of the post-glacial climatic deterioration some few centuries (about 850 B.C.) earlier than Sernander. It is possible that the deterioration set in earlier in the South.

The big task of today is to connect the post-glacial history of North America with that of Europe. If we get the evidence of a period of greater warmth and a subsequent deterioration of the climate in North America, are they or are they not contemporaneous with these periods in Europe?

THE PERIODS OF LATE QUARTERNARY TIME IN SWEDEN

	Geochronological acc'd'g. to DeGere	Evolution of the Baltic acc'd'g. to Munthe		Climatic periods acc'd'g. to Sernander	History of vegetation of Southern Sweden acc'd'g. to L. von Post	Archaeological periods acc'd'g. to Montelius	
1900							
1000	Post-glacial time	Mya time		Sub-Atlantic period	Time of beech and spruce forests	Historic times	
Birth of Christ						Iron age	
1000		Limnaea time					
2000		Litorina time	Post-glacial warm period	Sub-boreal period		Bronze age	Post-glacial climatic deterioration
3000				Atlantic period	Time of mixed oak forests	Stone age	
4000						Stone cist period	
5000						Passage Grave period	
6000		Ancylus time		Boreal period		Dolmen period Round age period	Maximum of the post-glacial land depression
7000	Fini-glacial time	Yoldia time		Sub-arctic period	Time of pine and birch	Older stone period Epipalaeolithical period	Fini-glacial climatic improvement
8000							
9000							The ice border at the middle Swedish end-moraines
10,000	Gothi-glacial time	Time of Baltic ice sea		Arctic period			
11,000					Time of the Dryasflora		
12,000	Dani-glacial time						The ice border in Skåne

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DYNAMIC FACTORS AFFECTING AQUATIC VEGETATION[1]

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In considering the causes affecting the distribution of aquatic vegetation, emphasis has usually been laid principally upon the factors which distinguish this type of vegetation from the terrestrial (that is, the predominance of water and its concomitant effects upon light intensity, temperature, and evaporation). Successional phenomena among aquatic plant communities have been usually regarded as chiefly biotic in origin. Other possibilities are, however, worth more attention than they usually receive, namely, that aquatic plants, like terrestrial ones, are largely influenced by edaphic factors, and that in consequence the distribution and successions among such plant communities are liable to be affected by factors of physiographic origin, which control the character of the soil. It is the object of this paper to indicate the methods and principles which have been employed in considering these possibilities and to illustrate them by reference to the distribution of submerged vegetation in some of the larger English lakes.

The lakes to which reference will be made are 11 in number and are mostly of similar initial size—2 to 5 miles, or else divided into basins of this size. They are useful as a series because they lie on rocks of very similar chemical composition. They are, moreover, all of glacial origin and of the type known as rock basins. The shores of these lakes were originally composed either of rock, of morainic boulders, or of a boulder clay, which usually under water becomes very greasy, tough, and impervious. Plant colonization would be, therefore, very slow until the shore line became modified in such a way as to form a penetrable substratum. While this initial difficulty would not apply in cases where the shore line was of soft material, the principles governing the subsequent development of vegetation appear to be of wider and more general application. Two sets of factors require recognition, (1) the forces altering the shores and (2) those affecting the lake as a whole.

In the first place, every shore is modified by wave action. This tends to cut out a terrace along the shore line, leaving the coarser materials along the terrace and removing the finer materials into deeper water. Further, along every exposed shore, a well marked littoral current or drift tends to be set up parallel to the shore. This drift slows down wherever it passes into deeper water and deposits silts. Hence bays and holes tend to be silted up while points and shallows tend to be swept clean. Moreover, the silts are deposited in definite

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 20, 1926.

order, the coarser first, usually in shallower water, the finest usually only in deep or comparatively stagnant water. Silt zonation along lines of silting is thus one of the most characteristic features of lake floors. It is not surprising to find that the distribution of rooted vegetation shows marked correlation with the distribution of different qualities of sediments. The evidence on which this correlation is based has been discussed at length in previous publications (Pearsall '18, '20, '21). For our present purpose it is only necessary to summarize the more general results of the detailed surveys in the English Lakes. There it is usual to find *Isoetes lacustris* L. on slightly silted stones or little altered boulder clay; *Nitella opaca* Ag. on more abundant silts—their quality being of minor importance; *Potamogeton perfoliatus* Wulf. and *P. praelongus* Wulf. on abundant, finer sediments; *Juncus bulbosus* L., f. *fluitans* Lam. on abundant, coarser sediments; while such species as *Naias flexilis* R. and S., *Hydrilla verticillata* Casp., and *Callitriche autumnalis* L. appear to occur only on the finest and most abundant sediments.

When comparable soil analyses are obtained, it appears that soils from areas where silting is rapid are richer in available potash, and usually in phosphates and nitrates than soils from areas where silting is slow (Pearsall '20). Further, the finer silts are richer in these substances than are the coarser ones. (This is no doubt due in part to absorption from the lake waters. Potash and phosphates are removed from water by soil suspensions, and in this removal the finest particles are the most effective absorbents.) It is probable that these chemical differences in the quality of the silts are one reason for the association of different plant communities with different types of sediments. Experiments have shown that when different species are grown side by side rooted in sand, in fertile garden soil or in fine lake soil, some species, notably *Isoetes Lacustris*, *Nitella opaca*, *Juncus fluitans*, and *Myriophyllum spicatum* are comparatively indifferent to the quality of the substratum, these being species growing in nature in localities where silts are either sparse or coarser. On the other hand, species like *Potamogeton perfoliatus*, *P. praelongus*, *P. pusillus*, *Naias flexilis*, and *Elodea canadensis* can only grow well on the fertile soils of the experiments, while in nature they are only found on the finer and chemically richer silts.

On the whole, therefore, it seems justifiable to assume that variations in the quantity and quality of silts very largely control the distribution of submerged vegetation, and to recognize some plants, especially *Isoetes* and *Nitella*, as characteristic of poorer and coarser silts, while other plants, like the submerged species of *Potamogeton* mentioned and *Naias flexilis*, are characteristic of the finer and richer soils.

Similar factors appear to control the development of vegetation in a lake taken as a whole. Quite clearly, if the shores are initially hard or unsuitable for plant life, erosion and sedimentation gradually improve the shores as plant habitats. In any case, however, the tendency must be for the shores to get overlaid by silts, because these are brought into the lake from the surrounding country. These silts would tend to improve the shores as plant habitats. In the case of the English lakes this process develops in stages. Glacial action

left the lakes and their surroundings essentially rocky. In the post-glacial period they have naturally tended to get silted. The sedimentation has, however, proceeded at very different rates in different lakes, chiefly because the underlying rocks vary in their hardness. The result of this has been that lakes lying on the harder rocks have remained comparatively unchanged (that is, rocky) but where the rocks are less resistant, the lakes have tended to be more silted. The point must, however, be emphasized that these extremes are stages in a post-glacial development, since they are due to the same change proceeding at different rates. We can, therefore, regard the rocky lakes as being relatively primitive, and the silted lakes as being more evolved (Pearsall '21). One other point must be noticed, that as the lake becomes more evolved, the sediments as a whole tend to become finer. This may be seen by comparing the relative transparencies of the lake waters, for the silted lakes have more turbid waters, not only because sediments are more abundant, but also because they are finer, and can therefore remain suspended for a longer time. Further, mechanical analyses of muds from comparable stations in different lakes agree in showing the rocky lakes to have coarser sediments (Pearsall '21), while, finally, the streams discharge into rocky lakes over sandy or gravelly deltas, but over muddy or silty deltas into silted lakes.

Now if our preliminary observations upon the relation between types of silts and types of vegetation are correct, it should follow that the vegetation in silted and evolved lakes should be conspicuously richer in the species characteristic of the finer and richer sediments, especially for example in the *Potamogeton* spp. as contrasted with *Isoetes* and *Nitella*.

TABLE 1. CLASSIFICATION OF ENGLISH LAKES ON THE BASIS OF THEIR PHYSICAL CHARACTERS AND ROOTED VEGETATION

Lake	Drainage System per cent cultivable	Lake Shore* per cent Rocky	Relative transparency of water	Per cent of submerged vegetation		
				Isoetes	Nitella	Potamogeton†
Wastwater	5.2	73	9.0	49	36	½
Ennerdale	5.4	66	8.3	35	48	½
Buttermere	6.0	50	8.0	40	40	1
Crummock	8.0	47	8.0	48	26	2
Hawes Water	7.7	25	5.8	5	71	5
Derwentwater	10.0	33	5.5	31	42	6
Bassenthwaite	29.4	29	2.2	42	3	3
Coniston	21.8	27	5.4	34	9	30
Ullswater	16.6	28	5.4	34	15	35
Windermere	29.4	28	5.5	9	40	38
Esthwaite	45.4	12	3.1	2	26	56

* To a depth of 30 ft.
† Including *Naias* and *Elodea* in small quantities.

The data given in the above table show that there are, in fact, remarkable general parallels between the physical characters of the lakes and their rooted vegetations. It is unquestionably true that the rocky lakes have a vegetation composed chiefly of *Isoetes* and *Nitella*, while as silts become more abundant there is a corresponding increase in the proportion of *Potamogeton*. The less

abundant constituents of the floras change also. *Myriophyllum spicatum* and *Juncus fluitans* tend to be associated with *Isoetes* and *Nitella*, while in the silted lakes, *Naias flexilis*, *Elodea canadensis*, with allied species are more abundant. Since the silted lakes are to be regarded as a later stage of the development of a rocky lake it follows that these differences in vegetation are to be regarded as a plant succession.

The general evolution of the lake basin in the manner indicated undoubtedly produces changes in the composition of the lake waters also. Soils or silts suspended in water tend to remove substances from solution (for example, potassium) and to give up other substances to the solution. For example, when potassium is absorbed, an equivalent amount of calcium (or of other bases) will be liberated. Hence the K+Na/Ca ratio will alter as silts become more abundant. Again, more silt in suspension offers greater opportunity for the solution of silica and hence more of this substance will tend to be present in the waters of the silted lakes. Since more silt also produces more vegetation and since the weathering which produces silts will also produce soil in the surrounding country, and hence also more vegetation there, the development of the lake basin will be associated with the appearance of more organic matter in the lake water and also more of the products of organic decay, such as carbon dioxide, carbonates, and nitrates. In the case of the English lakes, there is therefore a close parallel between the physical classification of the lakes and their classification on the basis of their water analyses. The silted lakes have a lower K+Na/Ca ratio, and more carbonates, nitrates, and silica in solution.

TABLE 2. WATER ANALYSES OF LAKE WATERS* (AS PARTS PER MILLION)

Lake	Potash and Soda	Lime	$\frac{K+Na}{Ca}$	Silica	Carbonate	Nitrate	Organic Matter
Wastwater	13.1	2.4	5.4	1.4	3.9	..	4.3
Ennerdale	7.3	2.4	3.0	1.4	2.4	...	5.6
Buttermere	15.4	2.8	5.5	1.4	3.2	...	6.5
Crummock	15.2	4.0	3.8	1.4	2.4	...	8.0
Hawes Water	12.3	4.0	3.1	2.8	7.1	trace	4.3
Derwentwater	7.7	7.5	1.0	2.1	3.1	trace	2.4
Bassenthwaite	8.3	7.9	1.05	2.8	5.7	.4	6.1
Coniston	11.2	4.8	2.3	2.8	8.5	.05	7.1
Ullswater	10.6	5.6	1.9	4.3	7.8	1.1	11.4
Windermere	5.4	10.1	0.53	3.3	6.4	1.4	10.8
Esthwaite	10.4	9.5	1.1	3.0	7.6	2.0	13.8

* Data from comparable samples with the lakes at summer water level.

The importance of these facts lies obviously in their bearing upon the composition of the phytoplankton, which must derive its nutriment entirely from the lake waters. Estimation of the composition of the phytoplankton is not easy because it varies greatly from season to season and from year to year. The following data are based on ten comparative samples from each lake taken as spring, summer, and autumn collections over a period of three years (Pearsall '25). The classification gives the average percentage composition of the phytoplankton as based on these samples and also the dominant and sub-

dominant species. All species forming at any time more than 66 per cent of the whole phytoplankton are regarded as being dominant at times (d), while sub-dominants (sd) at times form more than 33 per cent of the phytoplankton. Either of these methods of classification gives a similar result—that the rocky lakes have a phytoplankton in which green algae (particularly desmids) are at times dominant, while the silted lakes have a dominant diatom plankton, though blue green algae also become abundant. In intermediate lakes, *Dinobryon* is also common. It is worthy of remark that the contrast in the plankton types appears also to have a physiological basis. The algae of the rocky lakes are chiefly producers of carbohydrates, like starch, as in the case of the green forms, *Dinobryon* and *Peridinium*. The algae of silted lakes are chiefly fat producers, for example, diatoms, blue green forms, and *Ceratium*. A survey of a wider body of evidence (Pearsall '21, '22, '24) suggests that these differences in the phytoplankton are correlated with the chemical characters of the lake waters.

TABLE 3. AVERAGE PERCENTAGE COMPOSITION OF THE PHYTOPLANKTON

Lake	Green Algae	Diatoms	Starch Producers	Fat Producers
Wastwater	80	14	85	14.5
Ennerdale	79.5	15	83	16
Buttermere	40	15	82	18
Crummock	29	25	62	37
Hawes Water	24	30	39.5	60
Derwentwater	10.5	44.5	48.	51
Bassenthwaite	37.5	60	38.5	61
Coniston	31	45	31	68
Ullswater	7.5	87	10	89.5
Windermere	16	60.5	18.5	79.5
Esthwaite	9	33	13	86.

TABLE 4. PHYTOPLANKTON TYPES AT TIMES DOMINANT (d) OR SUB-DOMINANT (sd)

	Staurastrum spp.	Green spp.	Dinobryon	Tabellaria	Asterionella	Melosira	Coelosphaerium	Anabaena	Oscillatoria
Wastwater	d	sd							
Ennerdale	d	sd							
Buttermere		sd	d	sd					
Crummock		sd	d	sd					
Hawes Water			d	sd			sd		
Derwentwater			d	sd	d			sd	
Bassenthwaite		d		sd	d				
Coniston*				sd					
Ullswater				d	d				
Windermere				sd	d	sd	sd	sd	
Esthwaite				sd	d	sd	d	sd	d

* Phytoplankton very scarce—probably owing to mine washings.

Briefly summarized, the results of this survey permit the recognition of two main stages in the post-glacial development of vegetation in rock basin lakes of this type. These two stages are, in outline, as follows, fuller details being given elsewhere (Pearsall '21).

	Primitive	Evolved
Physical type	Rocky	Silted
Submerged and rooted vegetation	Isoetes and Nitella	More Potamogeton spp. with Naias, Elodea, etc.
Phytoplankton	Green algae, Desmids, and often Dinobryon	Chiefly Diatoms with more blue green algae
Fauna	Chiefly trout	Pike and Perch abundant

The recognition of these stages permits a suggestion of considerable interest—that the primitive lakes tend to contain primitive types of vegetation, while evolved lakes contain more highly specialized forms. Thus *Isoetes* and *Nitella* are both members of very primitive groups of plants of Lower Carboniferous or still greater age—while the Naiadaceae are of comparatively recent origin. Similarly the diatoms are geologically recent forms and it can hardly be doubted that they have been derived from some forms of green algae. So far as our knowledge of the fauna permits any conclusion, the same general principle seems to apply—that the primitive lakes contain the more primitive forms. It is not, however, desired to push this suggestion further, until similar features are recognized elsewhere.

Of more general application is a second principle, that the relative importance of the physiographic factors appears to decrease as the aquatic succession progresses. In the widest and most logical sense, an aquatic plant succession develops until a stable terrestrial plant community is formed (Pearsall '18a). Now as such a sequence matures there is a general increase in the organic content of the substratum and also a decrease in the rate of silting as the lake floor rises towards and emerges above the water level. These effects are usually very well summarized in peat sections of old lake bottoms—where the old subaqueous horizons are chiefly inorganic, while the emergent and marsh levels become increasingly organic as the soil level rises above that of the water. In England, these conditions finally result in the formation of moor vegetation and peat, which are practically independent of the ground water and silting. The general sequence, therefore, as shown in old lake beds, is a succession in which change is at first associated with silting almost entirely, and in which this factor becomes less and less important, until finally change is almost entirely due to accretions of dead vegetable matter. The relative importance of the silting and biotic factors, therefore, appears to vary very considerably at different stages in the plant succession.

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FACTORS CONTROLLING THE DISTRIBUTION OF SPECIES IN VEGETATION[1]

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There is considerable uncertainty in present day ecology in reference to such concepts as "habitat," "habitat factors," "ecological factors," etc., as well as to the amount of correlation between vegetation and habitat. Different authors use these terms in very different senses. Some believe in an almost complete parallellism between vegetation and habitat, while others regard this parallellism as being incomplete. Further discussion of this problem seems, however, of little use as long as ecologists have not arrived at a general agreement on the fundamental concepts just mentioned. The scope of the present paper is to make a few suggestions on the best use of "habitat" and related terms; to suggest a more detailed terminology for the different factors contained in "habitat," in its widest sense, as well as for the other groups of factors influencing the distribution of species in vegetation; and finally, to give a summary of my own opinion of the relative importance of all these factors.

I have tried to free myself from the traditional classification of "habitat factors" into "edaphic," "climatic," "biotic," "historic," etc., as coordinated groups. I believe this classification is too illogical to be used with advantage, as the different groups do not exclude each other. Thus edaphic, climatic, and biotic factors are often historical at the same time, while an edaphic factor is often of biotic origin, etc. Furthermore, those concepts are far too heterogeneous for our present needs. Especially does the "biotic" group comprise factors of very different type, and many that have very little in common.

The term habitat is used in present day ecological literature in two quite different senses: (1) In the sense of the recommendations made at the Congress at Brussels, that is, as somewhat mystical comprehensive of "der Gesamtheit der an einer geographisch bestimmten Lokalität wirkenden Faktoren, soweit sie die Pflanzenwelt beeinflussen." (2) In the sense employed by Ragnar Hult (1881), Fries (1913), Du Rietz, Fries, and Tengwall (1918), Gams (1918), Nordhagen (1919), etc., that is, as a purely geographic concept. I regard this divergence in use of the term habitat as one of the main causes of the luxuriant polemics in current ecology. I think the second alternative is better suited to be generally accepted by ecologists, and I would like to define the habitat simply as "the life medium of any plant or plant community."

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 20, 1926.

As understood here, the habitat is composed of different parts. Most plants live partly in the air, partly in the soil; for many plants (aquatics) water is an additional life medium. Air, as well as soil and water, is a rather complex part of the habitat and may be divided into several factors. These factors are often named "habitat factors"; for the sake of clearness, I prefer to name them "habitat-composing factors." The factors of the atmosphere may be named conveniently "climatic factors," those of the hydrosphere and lithosphere "edaphic factors." Thus rain-water and snow are climatic factors only so long as they are in the air; after they have reached the ground, they become edaphic factors. The origin of the different factors has no influence upon this classification, the habitat and its factors being regarded as an accomplished fact.

The habitat, however, may also be regarded from a causal point of view as the product of the "habitat-producing factors." By many authors these factors are simply named habitat factors. They may be grouped in the following way:

A. Actual factors.
- I. Abiotic.
  - a. Climatic, that is, factors of the free atmosphere.
  - b. Edaphic, that is, the inorganic factors-complex of the lithosphere and the hydrosphere.
- II. Biotic.
  - a. Non-antropeic, that is, not influenced by man.
    - 1. Phytobiotic.
      - α Climatic, that is, shadow, wind-shelter, increased humidity, etc., in the lower layers of the forest.
      - β Edaphic, that is, factors of the lithosphere and the hydrosphere caused by vegetation, for example, the formation of humus, increased soil humidity, etc.
      - γ Pyric,[2] that is, factors caused by forest fires or grass fires, etc.
    - 2. Zoobiotic, for example, the influence of animal excreta, carcasses, etc.
  - b. Anthropeic,[2] due to the influence of man.
    - 1. Direct, that is, industrial gases, irrigation, manuring, etc.
    - 2. Indirect, the same groups of factors as were included in the non-anthropeic biotic factors.

B. Historical factors, with all of the same groups as in the "actual" factors.

Having defined the habitat concept and having analyzed the different kinds of habitat-composing and habitat-producing factors, I think the time has come to analyze the degree of correlation between habitat and vegetation. It is quite clear that the distribution of species in vegetation does not depend solely upon the habitat in the sense here used, neither upon the habitat-composing nor upon the habitat-producing factors, but upon the habitat together with several other groups of "species distributing factors." The distribution of species

[2] These terms have recently been proposed by Nichols.

upon a certain spot during a certain period is controlled by the following groups of species-distributing factors:

I. The nature of the habitat at the beginning of the period concerned.

II. The distribution of species upon and in the vicinity of the spot concerned at the beginning of the time period (distribution factors, being the product of the history of all factor groups).

III. The supply of dispersal units (seeds, etc.) of each species during the period concerned (dissemination factors).

This supply is controlled by the following factor-complexes:

1. The factors of the above mentioned group II.
2. The dissemination-organization of each species.
3. The dissemination agencies available, such as animals, running water, wind.
4. Chance, a factor of paramount importance, especially emphasized by Palmgren.

IV. The strength of each species in competition on the spot concerned, and the ability of each species to transform the habitat (competition factors).

The competition factors are the product of the following groups of factors:

1. The absolute habitat-amplitude of each species.
2. The relative vitality of each species in the different parts of its absolute habitat-amplitude.
3. The life-form of each species.

These groups of factors, on the other hand, are to a very large extent a product of the history of the species and plant communities concerned, since both habitat-relations and life-form of species have developed under the strong influence of their earlier habitats and of the plant communities in which they have lived and competed.

V. Animals, man, and plant parasites directly interfering in the competition.

VI. The time elapsed after the beginning of the period concerned.

Only when all of the groups II–VI are constant is there a simple causal relation between vegetation and habitat, as believed in by many authors. But in most cases those groups of factors may be as variable as the habitat, the true causality of vegetation being thus impossible to determine without considering all those groups of factors.

Of course we may have the degree of parallelism between vegetation and habitat increased by giving to the habitat concept a wider sense, that is, by including in the habitat some of the other groups of species-distributing factors, as proposed by the Congress of Brussells. But is it really possible to include all these groups in the habitat concept, as must be done before an absolute parallelism between vegetation and habitat can be attained? I believe not. Anyhow, the attitude of the believers in this absolute parallelism towards this question must be clearly declared before a continued discussion of the real causality problems will be possible.

SUCCESSION: THE CONCEPT AND ITS VALUES[1]

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If we take succession to mean, in the broadest general way, the sequence of populations on any spot or unit of the earth's surface, we must recognize at once that the concept can be given useful scientific significance only if we can trace in such sequences certain uniformities which we can make the subject of investigation, comparison, and the formulation of laws. Thus changes in the vegetation of an area which resulted from a series of events unconnected with one another could not be considered a successional series in any useful sense, for it would have no coherence in itself nor could it be significantly compared with another series elsewhere.

Let us then confine our attention to those change-sequences which result from *connected* series of events and may thus be considered as wholes. Here it seems we are bound to draw an immediate and primary distinction between, on the one hand, those successional series which are on the whole caused by factors external to the changing populations and on the other, those which are primarily determined by the effects of the populations themselves on the habitat and the reactions of the habitat on the populations. In the first class we have successional series resulting from gradual changes of climate, for example, when broad-leaved forest is replaced by coniferous forest and coniferous forest by tundra on the gradual approach of an ice sheet, or when increasing aridity causes a replacement of forest by desert or semi-desert vegetation. Again, we have the successions brought about by geodynamic factors, such as erosion and deposition, which, if they are not so violent as to destroy the vegetation of an area altogether, may progressively alter the conditions of the substratum so as to force a change in the existing vegetation—the disappearance of existing species and the appearance of others in their places. Besides the mechanical and physical changes brought about by such agencies as erosion, deposition, and eluviation, and their effects upon soil water content, we have chemical changes in the substratum such as those caused by progressive leaching, with its progressive alteration of soil reaction and of the quantities and proportions of the effective ions presented in the soil solution.

All kinds of successional factors have this in common, that they are external to the plant population—they force change upon it from the outside. But in so far as the factors themselves act continuously and progressively for a con-

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 20, 1926.

siderable time—in so far, in fact, as they represent a connected change-sequence of events affecting vegetation, a corresponding progression may be traced in their working, and the changes they bring about in vegetation are properly regarded as successional changes. Such successions are often called climatic or physiographic successions, and quite suitably when climatic or physiographic factors are dominant in effecting the successional change.

Sharply contrasted with such external factors are those which depend primarily upon the activities of the plants themselves, the effects of these upon the habitat, and the reactions of the modified habitat upon the plant population. The leading features of the primary successions leading up to mesophytic forest in the temperate regions of the northern hemisphere are too well known to need more than the briefest mention. The activities of the pioneers on a rock face or talus slope, the gradual disintegration of the crude mineral substratum, the increase of humus and of water content, the deepening of the soil. the establishment of woody plants, the shading of the soil, the increase of air humidity, the competition of individuals and species, and the gradual evolution of the stable or relatively stable "climax community," these phenomena of the so-called "xerarch" succession have often been described, and in their broad outlines are familiar to all. Equally familiar is the "hydrarch" succession, so different from the "xerarch" in its earlier stages, but leading to an essentially identical climax, and also in the main primarily determined by the activities of the plants themselves.

It is true, of course, and must never be forgotten, that actual successions commonly show a mixture of these two classses of factors—the external and the internal. For instance, in a climate with a high rainfall the factors of eluviation and leaching are operative side by side with the accumulation of humus and increase of shade and atmospheric humidity in the development of climax forest. In the hydrosere on the edge of a lake the raising of the soil level may be brought about by silting as well as by the accumulation of organic debris; the two factors may be present in various proportions and the resulting plant populations may differ accordingly. The colonization of a talus slope may be continuously affected by fresh accretions of talus, which modify the actual succession from that which would occur if the original talus surface were stable and were left to the activities of the plants alone. Nevertheless, a succession which is mainly determined by the activities of the plants themselves in modifying the habitat is clearly quite a different sort of phenomenon from one which is mainly dependent on external factors, and it is to the former alone that Clements' conception of the climax community as an organism—or, as I prefer to call it, a *quasi-organism*—and of its development as an organic development, can be rightly applied.

I do not propose to enter into any discussion of the justification of this view of vegetation as an organism or quasi-organism, because I have dealt with the subject elsewhere in some detail.[2] I need only say here that I do believe the

[2] Tansley, A. G. The classification of vegetation and the concept of development. Journ. of Ecol. 8: 1920.

analogy with the organism to be legitimate and useful if it is not pushed too far, and especially if we abstain from making illegitimate deductions. One point in the analogy to which I may call attention is that the mixture of external and internal factors in development to which I have just referred occurs also in the development of an organism. We all know that the major factor in the development of an individual organism is its specific hereditary equipment, primarily the stock of genes with which it starts its career. Its adult character is gradually unfolded as a result of the interaction of these with the forces of the environment. A sufficiently suitable environment must be present for the ontogeny to take place at all. An environment differing more or less from the normal will modify the ontogeny correspondingly, and may bring about marked changes and modifications in the character of the adult. Yet no one can doubt that the stock of genes is the main determining factor. Always, the two classes of factors, internal and external, are at work in every ontogeny as in every succession. But in the organic succession, as in the organism, it is the plants themselves, the total stock of species available, corresponding with the total stock of genes in the organism, that is the predominant factor in determining the character of the succession and the nature of the climax. Whether in the long run the environment can alter the genes by direct action, or whether it works merely by favoring the development of some genes and of some combinations of genes at the expense of others, as it allows the development in a succession, of some species and some combinations of species at the expense of others, is a question which goes, of course, far beyond my topic, even if our present knowledge admitted of a clear answer, which in my opinion it does not. But we must all agree that ontogeny and developmental succession alike depend here and now upon the genes and species actually available, with their actual current potentialities of response to the different factors of the environment.

At this point I shall permit myself a small digression on the function of analogy in science. We often hear that "argument from analogy" is likely to be fallacious. I do not think anyone can successfully dispute the proposition. But when it is used as a weapon to oppose all use of analogy in argument I think it wholly misses the mark. No one is justified in arguing that because a plausible analogy can be constructed between two series of phenomena, therefore the second series must have all the characters and qualities of the first series and must be governed by the same laws. But the construction of an analogy between two series of phenomena is often most helpful in enabling us to focus the phenomena in a new way, and thus to discover principles which might otherwise have remained unknown, to bring to light real relationships which give isolated phenomena a significance that would otherwise have been undetected. We cannot dispense with any apparatus of thought that may assist us in this task, which is after all the main task of science. The value of analogy to science is its heuristic value. Criticism, of course, can never be dispensed with.

To return now to developmental or organic succession: This type of succession has been called "biotic"[3] to distinguish it from the types brought about by changing climatic or physiographic factors. Though it is, of course, quite true that the predominant factors at work in developmental succession are biotic because they work through the plants themselves, I do not think the term is usefully applied in this way. If we call such a succession a biotic succession, we have no terminological distinction between the normal progress to the climax brought about by the action of the plants themselves on the habitat and the case—which is at least theoretically conceivable—of a succession brought about by *external* biotic factors, for example, the progressive action of animals. Here I know I run the risk of coming into conflict with those ecologists who like to consider the whole complex of organisms—animals and plants together—as consituting the natural ecological unit or biome,[4] as Clements calls it. But on practical grounds it seems to me essential to consider the plant communities separately, and to regard animal factors as external to them, just as we regard climatic, topographic, and edaphic factors as external. And I think we want a special term to distinguish this developmental succession, which is the normal typical phenomenon of vegetation the world over, except in the very driest and coldest regions, from those successions determined by external factors. With considerable diffidence I suggest the term *autogenic* (as opposed to *allogenic*) *succession.*

Let us now consider a few cases of actual successions in the light of what has been said.

First let us take the classical case of the succession on the Lake Michigan sand dunes made familiar to all ecologists 27 years ago by the work of Cowles.[5] Here we have, broadly speaking and neglecting minor features, 7 zones of vegetation:

(1) Beach communities.
(2) Fore-dune communities.
(3) Cottonwood dune community.
(4) Bunchgrass and heath and pinewood communities.
(5) Black oak forest.
(6) Mixed deciduous forest.
(7) Beech-maple (climax) forest.

The first 3 of these zones do not *necessarily* represent a succession of vegetation at all. If the physiographic forces at work within these zones, from the edge of the lake waters to the inner limit of the cottonwood dunes, are in equilibrium, that is, if the supply of sand from the lake shore and its removal inland are roughly balanced, these zones will maintain their general position, though shifting in detail here and there. If, however, the supply of sand exceeds its removal a succession will be initiated: the waters of the lake will be dammed

[3] Cowles, H. C. The causes of vegetative cycle. Bot. Gaz. **51**: 161–183. 1911.

[4] Clements, F. E. Plant indicators, Carnegie Inst. 1920.

[5] Cowles, H. C. The ecological relations of the vegetation on the sand dunes of Lake Michigan. Bot. Gaz. **27**: 1899.

back, by the accumulation of sand bars in the shallow water near the edge of the lake, the land will advance on the lake, beach communities arise on the area primarily covered by water, fore-dune communities will arise on the area formerly occupied by beach communities, and cottonwood dunes will arise on the area formerly occupied by shallow pools or flat wet tracts of sand. The factors at work in bringing about these changes are predominantly physiographic, though the nature and capacities of the species of plants involved are naturally essential to the phenomena—as they always must be. The succession is a physiographic succession. The plant communities themselves are what Crampton called *migratory*,[6] that is, each can settle only in a habitat provided for them by a particular phase in a series of physiographic changes. The communities have no direct connection with one another, only an indirect connection through the series of physiographic changes. There is no gradual alteration of the soil, no competition between species.

The low dunes formed by *Calamovilfa*, by the willows, poplars, and sand cherry arise quite separately from the strand plants and would doubtless exist if strand plants were absent altogether. The cottonwood dunes, again, arise quite independently of the fore dunes. The cottonwood seeds germinate in shallow depressions where the ground water stands round about the level of the surface of the sand, and the seedlings or saplings, catching the large quantities of blowing sand, begin to accumulate heaps of sand which form the starting points of the great main dunes. Owing to its power of rapid growth and its capacity for unlimited formation of adventitious roots in the damp sand below the air-dry surface, the cottonwood can often keep pace with the growth of the dune, and dunes of great height are capped with the living and flourishing tops of these trees.

The cottonwood dunes do not, however, form an actual basis for the subsequent stages of succession. The subsequent vegetation never colonizes them unless they are stabilized by the formation of new high dunes on their lakeward side. The cottonwood dunes remain for a longer or shorter time before they are destroyed by wind erosion, or they gradually travel inland by continual accretion of sand blowing over the top of the dune and sliding down the landward face. On the low ground on the landward side of the more stable high dunes a different vegetation arises—either a bunchgrass community, or a heath community dominated by *Arctostaphylos uva-ursi*, and followed by juniper and pine. From this point onwards the succession is a true development, an autogenic succession, through black oak, and mixed deciduous forest to the climatic climax of beech-maple forest. At some spots on the coast, presumably because the wind and sand factors are not so intense, the heath vegetation follows directly on the dune grasses bordering the lake beach. Where the blowing sand factor is very intense the organic succession initiated by the bunchgrass or heath phase requires the shelter of the high dunes before it can make a start. The outer zones of the Lake Michigan sand shore zonation—the beach

[6] Crampton, C. B. The geological relations of stable and migratory plant formations. Scottish Botanical Review **1**: 1–61. 1912.

vegetation, the fore dunes, and the cottonwood dunes—are moulded by physiographic factors which are irrelevant to the subsequent development of climax forest, though the protection of the big dunes is necessary to give the requisite initial conditions in places exposed to large quantities of violently blowing sand. I think that these earlier phases which thus may or may not form an introduction to the main sere should be separated from it under some such name as the *prosere*.

Exactly the same is true of the simpler dune succession on the West European sandy coasts. Here we have a beach community of such plants as *Cakile*, *Salsola*, and *Atriplex*, with minute fore dunes formed by *Arenaria* (*Honckenya*) *peploides*, followed in many places by bigger fore dunes formed by *Agropyrum junceum*. The main range of dunes is dominated by *Ammophila* (*Psamma*) *arenaria*, and, unlike the cottonwood dunes of Lake Michigan, these form the actual basis of the subsequent succession, which leads to *Calluna* heath, or to scrub, and in some places (though not in England) to climax forest. Here I should call the beach community and the fore dunes (which may or may not be present) the *prosere*, the series from the *Ammophila* dunes to the climax the *autogenic main sere*.

Let us now turn to another type of succession which certainly differs from the typical autogenic sere, I mean the type in which the communities which develop do not prepare the soil for those which follow. A good example of this kind of succession is the glacial moraine succession described 10 years ago by Cooper[7] from the Mount Robson region, British Columbia. Here on the youngest moraine were found a very few scattered individual plants of *Epilobium latifolium*, *Castilleia pallida*, *Saxifraga aizoides*, *Lychnis apetala* and a seedling of *Picea Engelmanni*. On an older moraine there was a definite community of creeping shrubs and herbs, the *Dryas octopetala-Arctostaphylos rubra* community. There were also present occasional willows and very occasional spruce seedlings. On a still older moraine the *Dryas-Arctostaphylos* community dominated large areas, but was in competition with another community made up of shrubs, among which several species of *Salix* were the most important, while *Betula glandulosa* was occasional, increasing as the community grew older. In addition, frequent young spruces were present, the largest being 6 feet high, together with a few seedlings of *Pinus albicaulis*. The oldest moraine of the series was covered by an apparently open forest in which *Picea Engelmanni* was predominant, while *Pinus albicaulis* and *Abies lasiocarpa* were occasional. The spruces were old, up to 116 years, but did not reach more than 30 feet in height. They stood about 30 feet apart and between them was a dense growth of *Salix* and *Betula*. In occasional openings of this were patches of the *Dryas-Arctostaphylos* community, and some spots were still bare. We have in fact a young subalpine climax forest still in competition with both the earlier communities. All 3 have their beginnings upon the bare moraine, and grow side by side, the later stages not depending upon the earlier. The succession is "telescoped" in the

[7] Cooper, Plant Succession in the Mount Robson region, British Columbia. Plant World **19**: 1916.

sense that all the stages grow side by side, the earlier communities actually not attaining maturity before they are destroyed, or their existence is imminently threatened, by the later ones.

In such a succession—and numerous similar cases exist—the organic bond uniting the various successive communities is clearly not so close as in the typical autogenic succession. The later communities do not depend for the conditions necessary to bring them into existence on the earlier ones. The spruce forest is quite capable of starting on the bare moraine. The cause of the delay in its establishment is simply that its seeds take longer to arrive and the community takes longer to develop than the Salicetum, just as this, in its turn, takes longer to develop than the *Dryas-Arctostaphylos* community. The organic element in the relation between the successive communities is the power of those which arrive later and grow more slowly to suppress the earlier ones which develop more rapidly, because the later and slower growing communities are composed of bigger and longer lived plants. This kind of succession also requires to be distinguished.

The comments which I have made on the different natures of various successions are not, of course, intended as anything approaching a full discussion or analysis of the subject, but are merely designed to show that such analysis is necessary if we are to get the full benefit of the successional standpoint of studying vegetation. It is not enough to distinguish successions as xerarch or hydrarch, mesotropic or xerotropic, and so on. We must recognize first that only connected changes are successional, and then consider the nature of the connection between the successional phases. The connection may subsist only between the external causes which lead to the changes, as in climatic and physiographic successions, or it may extend to the actual relation between successive plant communities. Of such autogenic successions, in which the predominant factors lie in the plant communities themselves, we must distinguish between the type in which the later communities are later because they come later and develop more slowly, the only connection with the earlier ones being that the later eventually suppress the earlier because they are composed of bigger plants, and the type in which successive communities depend for their initial development on modification of the habitat factors by the preceding ones—the most closely-knit type of succession.

Let us now turn to the actual value, or rather values, of the concept of succession in the study of vegetation. The value of every scientific concept is measured by the use to which it can be put, to its success in enabling us to focus phenomena more successfully, and thus to discover new starting points for fresh investigation. Almost everyone who has seriously applied the successional concept to the study of vegetation recognizes that it affords an indispensable framework for the communities which we distinguish in the field, that we cannot appreciate correctly the status of a community until we consider its place in the succession, its relation to the other communities in time as well as in space. So far from being a separate branch of vegetational study distinct from the study of communities as static units, it is the only sound foundation on which to base the systematic investigation of vegetation. Without it our knowledge of com-

munities remains disconnected and fragmentary, or is systematized in an artificial scheme, like our knowledge of species, without a natural system based on the theory of descent with modification. It is true that we want to know more than we can ever learn from the study of existing successions. We want to know how the communities whose sequences we now trace were originally built up, how these particular aggregates of species, and these particular life-forms, came into existence, and how they came to replace other aggregates and other life-forms in recent geological time. The whole subject of the origin of existing vegetation opens up before us, with its difficult problems, partly depending for their solution on exact and detailed knowledge, still very fragmentary though rapidly increasing in extent and certainty, of the migrations of species and their correlations with changes of climate; and partly concerned with the mode of origin of life-forms and of species themselves, about which we still know very little indeed.

But putting aside these larger questions, whose scope extends far beyond my topic today, we cannot but recognize that the study of existing successions is of the first importance in providing the most natural framework within which to classify our communities. This depends on two facts which no one can dispute. The first is expressed in the often quoted aphorism that vegetation, like all life, is dynamic; the second is that, given a particular complex of habitat factors maintaining itself sensibly constant in a given place for a significant time, the vegetation, that is, the totality of plant forms having access to the place, will progress towards a state of equilibrium both with the factor complex and within itself. This conception of climaxes of development gives us a number of fixed points from which to survey vegetation, and is of cardinal importance in the science. As I have shown in another place[8] different characters of these climax communities have been emphasised by different authors according to their special interests, but the fact of their existence no one doubts. I have upheld the view that the term *association* should be confined to the largest of these climax units, which are dominated and characterized by the same species, whatever the nature of the factors which hold the community in equilibrium, so that the community maintains itself sensibly constant so long as the factor-complex remains sensibly constant. I am well aware that this usage and its application are not free from difficulties. I do not think any system of vegetational nomenclature is, or ever will be, without these, because of the infinite variability of the constitutions and plasticities of plant species and of the combinations of habitat factors. But I do think it is a clear, practical usage, firmly based on the two facts I have recited, and corresponding well with the empirically determined combinations of species we meet with in the field.

There has recently been a tendency in some quarters to throw doubt on the validity of the concept of the climax, to argue that so-called climaxes are not eternal, are in fact probably always slowly changing, and that there is every gradation from very transitory communities to those which maintain themselves in a relatively stable condition for long periods. This is doubtless true, and

[8] Tansley, A. G. The classification of vegetation and the concept of development. Journ. Ecol. **8**: 131. 1920.

illustrates some of the difficulties of application to which I have referred, but it does not seem to be a valid argument against the use of the criticism of relatively stable equilibrium as the test of the communities which we should use as the foundation of our classification. After all, ecologists familiar with a region do in practice agree as to the communities which are relatively permanent under given conditions, though they may differ as to the *grade* of community to which such a term as *association* should be applied. The concept of the climax is so firmly based on universally admitted facts, and corresponds so well with empirically determined combinations of species that I cannot see how we can possibly abandon its use as the key-concept in the classification of vegetation.

Having established so much, we must, of course, recognize that our ultimate object as students of ecology is not to describe climaxes and successions but to analyze the cause which lead to them. And here we come upon the second great value of the successional standpoint. By focusing our attention of succession we are forced to attend first to change in vegetation, and secondly to the arrest of that change at certain points. We are thus naturally led to inquire how these things happen, and to study the forces which bring about the changes and their arrest. These forces, of course, include far more than the habitat factors in the narrower sense. They include the means of migration of constituent species, the extent of their plasticity, the details of their reactions to and their reactions upon the habitat, and the details of their interrelations—competition, protection, and so on. For all this sort of study the successional standpoint and classification are the best starting points, precisely because vegetation is dynamic, consisting of a series of actions and inter-actions. If we consider the vegetation of a region as a series of static entities, and proceed to inquire why such and such a community exists in such and such a place, and why it is constituted as we find it, we are at a loss where to begin and how to proceed. Lists of species and measurements of environmental factors may tell us little or nothing of value for the purposes of our enquiry. We do not know how to distinguish the significant from the unessential. But if we begin by considering the existing communities as members of developmental series, distinguishing those which are relatively permanent from those which are transitory, and tracing out the actual sequences and climaxes we find, we are at once brought into contact with the actual processes at work and can direct our studies of species and of habitat factors to the significant and crucial points.

One last point. We must always beware of hypostasizing abstractions, that is, of giving them an unreal substance, for it is one of the most dangerous and widespread of vices through the whole range of philosophical and scientific thought. I mean we must always remain alive to the fact that our scientific concepts are obtained by "abstracting from the continuum of sense-experience," to use philosophical jargon, that is, by *selecting* certain sets of phenomena from the continuum and putting them together to form a concept which we use as an apparatus to formulate and systematize thought. This we must continually do, for it is the only way in which we can think, in which science can proceed. What we should not do is to treat the concepts so formed as if they represented entities

which we could deal with as we should deal, for example, with persons, instead of being, as they are, mere thought-apparatuses of strictly limited, though of essential value.[9] Thus a plant community is an essential concept for the purposes of the study of vegetation, but it is, on the other hand, an aggregation of individual plants which we choose to consider an entity, because we are able to recognize certain uniformities of vegetational structure and behavior within the aggregation by so doing. A climax community is a particular aggregation which lasts, in its main features, and is not replaced by another, for a certain length of time; it is indispensable as a conception, but viewed from another standpoint it is a mere aggregation of plants on some of whose qualities as an aggregation we find it useful to insist. Again we pick out successional series (seres) from the kaleidoscopic process of changing vegetation, because we can thus put together and formulate certain uniformities of process which serve to consolidate and systematize our knowledge. These selective syntheses are essential to the progress of science, and the particular ones mentioned are of very great value, as I have tried to show, in the study of vegetation and of ecology. But we must never deceive ourselves into believing that they are anything but abstractions which we make for our own use, partial syntheses of partial validity, never covering *all* the phenomena, but always capable of improvement and modification, preeminently useful because they direct our attention to the means of discovering connections we should otherwise have missed, and thus enable us to penetrate more deeply into the web of natural causation.

It is the special credit of American ecology, and in the first place of the labors of Cowles and of Clements, followed by a host of gifted workers, that it laid stress upon this successional way of viewing vegetation at a critical epoch in the development of the science, and thus fertilized and vivified the whole study of ecology by a general mode of approach which has had the most far-reaching results, and whose usefulness is still far from being exhausted. In the application of the study of vegetation to practical problems of the utilization of land it is the successional point of view which is much the most fertile, and its great possiblities of practical usefulness, especially in "new" countries, are only now beginning to be fully recognized. No higher testimony to the usefulness and therefore to the validity of a scientific concept can be expected or desired.

[9] A good example of the hypostasization of an abstraction, exceedingly common 40 years ago, but now happily rare, is the treatment of the process of natural selection as if it were an active force, a sort of *deus ex machina* which always and everywhere modified species and created new ones, as a breeder might do with conscious design.

THE SUCCESSION POINT OF VIEW IN FLORISTICS[1]

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I. Introduction

Warming recognizes a sharp contrast between the ecological and floristic points of view in plant geography. In the treatment of any given region, authors mainly have taken the ecological viewpoint or the floristic viewpoint, in the one case considering the area from the standpoint of its associations and successions, and in the other case from the standpoint of its floristic composition.

From the floristic point of view the flora of a region is considered as to its origin and immigration. From this angle the plants of any given region may be divided into 4 classes, first, those that are strictly proper to the region and distributed more or less equally in all directions from that region; second, plants that are proper to the region under consideration, but which are at or very near the edge of their range so that they are distributed unequally in different directions from the region under consideration; third, plants that are discontinuous or disjunct; and fourth, endemic plants. In most regions the first category noted above is much the largest as to number of species. It is also the least interesting because it is the thing that is expected. The remaining categories are all of a special interest to students of floristics, because they have a very definite bearing upon the past history of species, and upon the migration of species both past and present.

The present paper is a successional study from the floristic angle. The associations of a given region (the Chicago region) are presented in their dynamic relations or order of development, and comparisons are made of the floristic characters of the various associations. Since the Chicago region was covered by Pleistocene glaciers, the present flora is entirely post-glacial. Successive waves of vegetation have passed northward over the Chicago region, the first having been an Arctic or tundra wave, the second, a wave dominated by sub-Arctic types; then followed plants of cold temperate character, such as now occur about Lake Superior; then came the deciduous angiosperms and forest types that still dominate in the Chicago region; finally there came types that are even yet more characteristic of areas to the south of Chicago. Each of the vegetation waves of the past has left relicts stranded in the Chicago flora of today, but these stranded relicts are much more characteristic of certain associations or stages of succession than they are of others.

[1] Presented before the International Congress of Plant Sciences, Section of Ecology, Ithaca, New York, Aug. 20, 1926.

The waves of vegetation noted in the preceding paragraph must not be supposed to have passed strictly northward over the Chicago region. Some of the waves may have come from the southeast and passed toward the northwest. Other waves may have come from the southwest and passed to the northeast. In general the waves from the southeast represent the more humid elements of the flora and the waves from the southwest the more arid elements. Similarly, with changes in humidity and aridity, waves may have passed over the region from east to west or from west to east, as well as from south to north.

II. An Ecological Study of Floristics in the Chicago Region

A. *The Beach-Dune Succession.* One of the most interesting of successions floristically is that of the Dunes. The beach is especially characterized by maritime elements (*Ammophila, Lathyrus maritimus, Cakile edentula, Euphorbia polygonifolia*, etc.), whose presence along a fresh water lake 1000 miles inland is difficult to explain. The foredune and the pine dune that immediately succeeds it have a flora of distinctly northern aspect and are rich in relicts of the vegetation waves that followed the ice retreat (*Salix syrticola, Pinus Banksiana, Juniperus horizontalis, Juniperus communis depressa, Arctostaphylos Uva-ursi, Populus balsamifera*, etc.). The next succession stage dominated by oaks, is largely characterized by plants of the present vegetation wave, however with some xerophytic western elements (*Opuntia, Castilleja sessiliflora*). The climax forest that follows the oaks also has plants that are mostly characteristic of the present wave, but more than any preceding stage, the climax forest is rich in recent invaders from the south (*Liriodendron, Cornus florida, Asimina, Stylophorum*, etc.). One of the noteworthy things in the pine or evergreen dune is the fact that not only are many northern species present, but that they also dominate over the other elements. The vegetation as a whole is much more northern than proper to the Chicago region.

B. *The Pond-Bog-Forest Succession.* Another notable succession is that from the pond through the bog to the climax forest. The pond and the pond margin have a cosmopolitan or circumboreal (as *Calla*) flora, but the sedge bog, the bog thicket, and the bog forest have a noteworthy congestion of relicts from the subarctic and later waves (*Larix, Betula pumila, Chamaedaphne, Andromeda, Salix* spp., *Cornus canadensis, Linnaea*, etc.). The climax in this succession has much the same composition as in the climax on the dunes. Both in the dune and bog successions the relicts characterize the earlier stages, while the new invaders especially characterize the climax forest. These relations probably may be referred to competition which is greatest in the climax and less in the early stages. Though the relict plants in these successions are not particularly in harmony with the present climate, they can none the less continue by invading new areas from century to century. In the dunes this is made possible by the continued advance of the shore line into Lake Michigan, making constantly new places for the occupation of the northern relict plants as they are crowded out by the invasion of the oaks and climax forests on the older dunes to the south. In the bogs the continued existence of the northern relicts is made possible by the constant

advance of the swamp flora into the ponds. In this instance, of course, there is a definite limit to the persistence of the northern relicts in the region for, after a time, all the ponds will be filled up and grown up to climax forest. The extinction, therefore, of the northern relicts in the bogs may be looked for before the extinction of the northern relicts in the dunes.

C. *Other Successional Series.* Northern relicts occur also in at least 3 other successions in the Chicago region, the Rock Canyon Succession, the Lake Cliff Succession, and the Spring-Fed Clay Ravine Succession. In these successions also competition is less keen in the early stages than after the invasion of the oak forest or the climax forest of the region. Amongst the northern relicts found in Rock canyons are *Campanula rotundifolia*, *Taxus*, and *Galium boreale*. A noteworthy relict of the Spring-Fed Clay Ravines is *Equisetum scirpoides*. A relict of the Lake Cliff is *Shepherdia canadensis*.

III. Southwestern Limits and Their Significance

One of the striking features of the Chicago region, as perhaps of other regions in the middle west of the United States, is the distribution of northern species at the southwest edge of their range. Their behavior there may be regarded as extremely significant of the present trend of migration either north or south or east or west, or a combination between 2 of these directions. The southwestern station of all of the following plants is probably in what may be called the Chicago region: *Thuja occidentalis*, *Betula lutea*, *B. alba papyrifera*, *Juniperus horizontalis*, and *Primula mistassinica*, as with other northern relicts, these occur in pioneer edaphic situations. Four possibilities present themselves in the climatic changes of a region, not only in the recent past, but also probably at the present time. The region may be becoming drier and warmer, or drier and colder; or it may be becoming wetter and colder, or wetter and warmer. It is probable that the most usual changes are represented by drier and warmer or wetter and colder. Probably at the time of the southward invasion of the ice sheets, the climate in the Chicago region, as of regions generally, was becomming wetter as well as colder. Similarly, with the northward retreat of the ice, the climate probably became drier as well as warmer. Therefore, at the time of glacial advance, the climax forest must have retreated southward, and at the same time it may also have advanced westward because of increasing humidity. The combination of these 2 movements would mean a general dominant movement in a southwesterly direction. During the period of glacial retreat, there was not only a northern advance of the climax forest types, but also probably an eastern retreat of climax forest types, so that in general the resulting dominant movement was probably in a northeasterly direction. If, at any time, the Chicago region became drier and colder, there would have been a retreat of the climax forest in 2 directions, east and south, with the resultant movement dominantly southeast; and if there is a period that becomes wetter and warmer, it means an advance of the climax forest in a northerly and westerly direction, the resultant dominant direction being northwest. The above conclusions show, therefore, that at the southwestern edge of its range, the behavior of a species, whether it appears to be gain-

ing or losing, indicates the trend of climatic change at the present; and the edaphic occurrence of these species may often yield important data also.

IV. The Disjuncts of the Chicago Region

Generally speaking, there are 4 categories of such disjuncts, those of eastern range; those of western range; those of southern range; and those of northern range. The disjuncts of northern range are much the more numerous and much the more readily explained. They have been referred to in preceding paragraphs and may be regarded as relicts that have been left behind in connection with a northward retreat of glacial ice and of the northern movement of the flora following this retreat. The disjuncts of eastern range are mostly maritime species, either of the dunes or of the swamps. The most important maritime disjuncts of the dunes are *Ammophila*, *Cakile*, *Lathyrus maritimus*, and *Euphorbia polygonifolia*. Among the maritime swamp disjuncts are *Triglochin*, *Utricularia purpurea*, *Hibiscus Moscheutos*, *Ranunculus Cymbalaria*, and *Juncus balticus*. It is not easily possible to explain the maritime disjuncts, but certain authorities have suggested the possibility that in some period of post-glacial time the sea has been very much nearer the lake region than it is at present. It should also be noted that these species find congenial habitats on the sandy lake shores and might, therefore, migrate from lake to lake.

The disjuncts of western range are of very fascinating interest. They consist of 2 definite sorts of elements. *Actinea herbacea* is a close relative of *A. acaulis*, a typical xerophytic pioneer in the Rocky Mountain Region and occurs in one local habitat near Joliet, Illinois and also in northern Ohio. It may have migrated eastward in some dry post-glacial (xerothermic) period and been generally eliminated in a succeeding period of increased humidity. Equally striking is the appearance in the lake region of humid elements of the Rocky Mountain flora, although these do not occur to any extent in the immediate neighborhood of Chicago. Among such plants are *Fatsia horrida*, *Lonicera involucrata*, and *Vaccinium membranaceum*. These elements which occur in mesophytic habitats would seem to point to a humid period in post-glacial times, during which they may have existed between the lake regions and the mountains in areas where they now are absent. Perhaps the most striking of our disjuncts are those of southern range, notably *Populus heterophylla*, *Aesculus glabra*, and *Styrax americana*. It is not easy to explain these southern disjuncts unless there has been, since the last ice epoch, a period that is warmer or wetter than the present. It is interesting to note that these southern relicts are found in the most favored spots of the regions where they grow, in this respect being quite in contrast to the northern relicts which inhabit pioneer situations that are unfavorable for the majority of plant species.

V. Endemics

The endemics may be briefly considered. Their significance is much like that of disjuncts. For example, *Sphaeralcea remota*, which is known to occur only on a small island in the Kankakee River, is closely related to certain western species

of the genus. It may be explained as perhaps recently derived from some parent form that once connected the Chicago region with the west. Other endemics that occur in the Chicago region are *Sullivantia Sullivantii*, *Synthryis Bullii*, and *Cirsium Pitcheri*. These endemics are edaphic pioneers. In addition, there is the remarkable endemic known only from a small area within the city of Chicago, *Thismia americana*, whose nearest known relative is in the East Indies. The occurrence of *Thismia* presents a situation of extraordinary difficulty.

In closing, the author wishes to make appeal for a general ecological study of floristic data in the hope of explaining many of the difficult facts of floristics by this means. It similarly would seem to offer a very interesting outlet for ecological investigation.

LA SPERIMENTAZIONE FORESTALE COME FONDAMENTO SCIENTIFICO DELLA SELVICOLTURA, CON PARTICOLARE RIGUARDO ALL'ITALIA[1]

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L'importanza che la sperimentazione ha, come base di una selvicoltura razionale, non richiede d'essere dimostrata. Ormai tutte le nazioni civili hanno compreso che la produzione forestale, come quella agraria, debbono larga parte del loro progresso e del loro incremento alla sperimentazione scientifica. In questa breve nota dunque non si tratta di dimostrare la necessità della sperimentazione forestale ma piuttosto di indicare in qual modo questa debba essere intesa ed organizzata, così che possa raggiungere i suoi fini.

Questo nostro studio partirà da considerazioni e rilievi di ordine generale per venire a particolari, riguardanti le direttive e gli scopi della sperimentazione forestale in Italia.

La produzione forestale ha una caratteristica, per la quale si differenzia notevolmente dalla produzione agricola: il lungo ciclo attraverso il quale essa si svolge. Questa lentezza della produzione legnosa, che riflette la lunga vita della foresta, è di per se stessa un grave ostacolo alla possibilità di trarre direttive precise dalla sperimentazione forestale, se non a termine molto lontano. Di qui due necessità:

(1) Che la sperimentazione forestale deve essere seriamente e solidamente organizzata, in modo da assicurare la assoluta e perfetta continuità delle ricerche attraverso lunghissimi periodi.

(2) Che la *sperimentazione* propriamente detta deve essere fiancheggiata e appoggiata dalla *osservazione* della vita e produzione dei boschi, osservazione che in molti casi potrà permettere la conquista di risultati positivi in tempo assai più breve e con maggiori garanzie che attraverso le esperienze istituite ex-novo.

Queste due caratteristiche fondamentali ci portano dunque ad asserire che la funzione di osservazione deve assumere un'importanza pari, se non superiore, a quella della *esperienza*, e che quest'ultima deve essere organizzata in modo continuativo.

La realizzazione pratica di questi principi può avvenire in modo assai diverso a seconda delle caratteristiche, delle esigenze e delle possibilità dei diversi paesi;

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca New York, Aug. 17, 1926.

ad ogni modo essa non può prescindere dalla necessità di differenziarsi a seconda dei vari oggetti della ricerca. Così ad esempio le ricerche di statistica e di economia forestale non possono essere intese ed organizzate alla stessa stregua di quelle biologiche. Infatti nel campo dell'economia e statistica forestale la *osservazione* ha il sopravvento assoluto sull'esperienza, e la osservazione stessa può essere affidata ad organi diversi della Stazioni sperimentali specializzate; così i servizi di statistica forestale possono essere collegati a quelli di statistica agraria, e gli studi di economia forestale quasi sempre non si possono scindere da quelli dell'economia agraria ed anche della economia politica.

Anche l'organizzazione internazionale della sperimentazione non può essere uguale per le ricerche statistiche e per quelle di biologia forestale e selvicoltura; così ad esempio è necessario unificare rigorosamente i metodi di statistica per addivenire a risultati veramente attendibili e confrontabili, mentre l'unificazione dei metodi nelle ricerche biologiche o colturali non è adottabile che per determinati campi di ricerca.

Il principale oggetto di attività per la stazioni sperimentali è appunto quello che riguarda la biologia, la selvicoltura e la tecnologia forestale. Su questa funzione fondamentale crediamo opportuno fare alcuni rilievi.

Anzitutto si deve asserire la necessità del decentramento della sperimentazione. In selvicoltura, più ancora che in agricoltura, è indispensabile eseguire osservazioni ed esperienze in campagna; questa è la sperimentazione essenziale, poichè quella di laboratorio (comprendiamo nella sperimentazione di laboratorio anche quella fatta in limitati appezzamenti di terreno o giardini sperimentali annessi alle stazioni) deve essere intesa solo come complemento ed ausilio. Anche nelle ricerche tecnologiche, in cui la sperimentazione di laboratorio ha una particolare importanza, non può trascurarsi quella di campagna, perchè, ad esempio, la conoscenza dei caratteri tecnologici di un legname ha scarso valore, quando non la si ponga in relazione al tipo di foresta dal quale proviene, alle condizioni di ambiente nelle quali è stato prodotto e così via. E, per fare un altro esempio, i caratteri di una resina—studiati in laboratorio—debbono essere messi in relazione con la tecnica dell'estrazione, con i caratteri biologici e colturali degli alberi che la producono.

Ma la sperimentazione di campagna, a sua volta, deve essere organizzata in modo da abbracciare il più grande numero possibile di quelle condizioni che interessano l'oggetto della ricerca. Questo carattere grandemente estensivo della sperimentazione forestale s'impone ormai oggi come una condizione indispensabile al conseguimento di resultati positivi e concreti. Lo sviluppo della nostra scienza ci ha ormai dimostrato che l'ecologia e la biologia debbono essere le basi della selvicoltura razionale; ora, lo studio dell'ambiente fisico (clima e terreno) non si può comprendere che come uno studio estremamente suddiviso nelle variabilissime condizioni ambientali, così da portare alla individuazione di quelle unità ecologiche che si presentano a determinare ed influenzare la vita e la produzione della foresta.

Resultati sperimentali ottenuti dopo anni e decenni in una data stazione, non possono essere estesi praticamente che a stazioni ecologicamente eguali,

epperciò il loro valore è tanto minore quanto più grande la varietà delle condizioni ecologiche che si presentano in un dato paese, nei casi concreti in cui tali resultati dovrebbero essere messi a profitto della selvicoltura pratica.

Così, ad esempio, lo studio sperimentale della provenienza del seme, delle proprietà e caratteri biologici e colturali delle diverse razze di una data specie, non può portare a risultati pratici di serio valore se non è organizzato in modo estensivo, con esperienze multiple. Ricerche sperimentali mirabili eseguite sulla provenienza dei semi da studiosi illustri come il Vilmorin in Francia, il Ciesldt in Austria e l'Engler in Isvizzera, se portarono, tra le prime, al resultato importantissimo di dimostrare il diverso comportamento delle razze di diversa provenienza, non poterono dimostrare il valore delle razze stesse che per le pochissime località dove le esperienze furono condotte. Noi non sappiamo quale sarebbe stato il resultato delle razze medesime, se fossero state sperimentate in altre località; ne consegue che il valore pratico di queste esperienze è molto scarso.

Perciò giustamente Schotte affrontò in Isvezia lo studio dell'importante problema col metodo estensivo: per il solo pino silvestre di provenienza svedese egli iniziò nel 1905 la serie delle parcelle di prova ed oggi la Stazione sperimentale Svedese controlla 450 parcelle sparse in ogni parte del paese. Quando potranno essere consacrati dal tempo i risultati di questa grandiosa sperimentazione, la selvicoltura svedese potrà veramente possedere linee direttive sicure in questo campo.

Ciò che abbiamo detto per il problema della provenienza vale anche per tutti gli altri problemi che hanno attinenza con la biologia forestale e la selvicoltura. Si deve poi osservare che il tempo necessario al conseguimento di resultati positivi in questo genere di esperienze è lunghissimo; perciò la simultaneità dell'impianto delle sperimentazioni multiple s'impone, ove non si voglia trascinare attraverso i secoli il lavoro sperimentale, da cui invece i selvicolturi attendono la soluzione di problemi di grande importanza pratica ed economica. In altre parole, se nella sperimentazione agraria—che ha ciclo breve—si può passare sistematicamente da una località all'altra per studiare sperimentalmente un dato problema, nella sperimentazione forestale non si può adottare lo stesso metodo e non si può neppure limitarsi a concentrare le esperienze in poche località.

Ma per organizzare la sperimentazione forestale in modo da interessare molteplici ambienti e da obbedire quanto più è possibile al principio della simultaneità, sono necessari ingenti mezzi finanziari e larga disponibilità di personale scientifico e tecnico. Infatti solo dove si sono verificate queste condizioni (per esempio in Isvezia in Finlandia ecc.) la sperimentazione forestale è stata avivata in modo conforme ai suddetti principi.

Ma dove, per un complesso di ragioni, questa organizzazione ideale non si è potuta raggiungere o si potrà raggiungere solo in un lontano avvenire, come orientare la sperimentazione? Noi non esitiamo ad asserire che, piuttosto di concentrare le esperienze in qualche arboreto o in poche limitate parcelle di prova, per seguirle con tutta la diligenza, è meglio dare una grande estensione

alle esperienze, anche se non sarà possibile raggiungere una cura minuziosa nel controllare il loro andamento. Man mano che il servizio sperimentale potrà perfezionarsi e migliorarsi, si intensificherà il controllo; ma intanto il tempo non sarà perduto, si saranno assicurati elementi preziosi alla futura sperimentazione e le linee essenziali dei resultati non potranno non rivelarsi egualmente. Un attivo servizio di osservazioni sistematiche potrà poi—come si disse—integrare con grandissima efficacia il lavoro sperimentale propriamente detto.

Certo è difficile dare un valore assoluto e generale a queste nostre asserzioni, in un campo così vasto e così differente da paese a paese, ma è certo che esse sono fondate per quanto riguarda alcuni rami importantissimi della sperimentazione, come ad esempio la biologia del bosco (studio delle formazioni vegetali, dei tipi di foresta, delle relazioni tra i vari elementi della formazione forestale e tra questi e l'ambiente fisico ecc.) studio dei climi e dei terreni, della provenienza del seme, della coltura di specie esotiche, della rinnovazione naturale, dei vari modi di governo e trattamento dei boschi, della tecnica dei rimboschimenti, ecc; studio della produzione legnosa e di quella di frutti, cortecce, succhi, resine ecc.

Anche lo studio dei metodi di assestamenti, in quanto essi non sono e non debbono essere che l'applicazione metodica di determinati procedimenti di coltura e trattamento del bosco, ma può sottrarsi alla necessità della moltiplicazione delle esperienze e delle indagini nel più grande numero possibile di località, anche se ciò riuscisse a scapito della minuziosità delle osservazioni.

Per ciò che concerne la scelta degli obbiettivi di studio, essa naturalmente potrà essere guidata dalle particolari condizioni ed esigenze di ciascun paese. Senza togliere alla ricerca scientifica quella libertà di azione ch'è una dalle sue perogative più belle, non si può negare che l'importanza della sperimentazione forestale sarà tanto più apprezzata dal pubblico competente e dalle autorità governative, quanto più la sperimentazione stessa sarà diretta a risolvere problemi di vasta e reale importanza pratica.

A questo intento, uno stretto collegamento degli Istituti sperimentali con le Amministrazioni forestali governative e con gli Enti che dedicano la loro attività alla selvicoltura, ci sembra necessario. Su questa via si sono già messe le Stazioni sperimentali di molti paesi; essa però nel passato non è stata sempre seguita, come dimostrano recenti esempi. Così ricordiamo che con decreto del marzo 1924 si dava una nuova organizzazione alla sperimentazione forestale della Baviera realizzando una intima unione fra le stazioni sperimentali ed i servizi forestali e disponendo fra l'altro che il personale forestale collaborasse con la stazione stessa per determinati gruppi di ricerche e di esperienze.

Questa collaborazione del personale forestale può avere i suoi inconvenienti e sopratutto può riuscire onerosa al personale stesso che deve dedicare ogni attività alle sue ordinarie mansioni; ha però l'inestimabile vantaggio di tenere sempre in diretto contatto della vita forestale vissuta il personale addetto alle

[2] L'attività del R. Istituto Superiore Forestale Nazionale dalla sua fondazione al 1924—Firenze, 1925.

sperimentazioni, offrendo ad esso sempre nuovi e preziosi argomenti di osservazione, di studio, di discussione.

L'organizzazione della sperimentazione forestale in Italia risponde precisamente ai concetti dianzi espressi.

Il compito della sperimentazione forestale fu affidato nel 1914 al R° Istituto Superiore Forestale Nazionale, fondato in tale anno a Firenze a scopo prevalentemente sperimentale, subordinatamente a scopo didattico. Perciò fin dall'inizio l'Istituto fu dotato di ben attrezzati laboratori e mezzi di ricerca e si avviarono così i primi studi tanto nel ramo dell'Economia e statistica forestale come in quello della biologia forestale, selvicoltura, assestamento e tecnologia. I risultati di tale sperimentazione svoltasi nel primo decennio di attività dello Istituto sono dimostrati nella collezione degli Annali dello Istituto stesso ed inoltre da apposita pubblicazione[2]. Non è quindi il caso di esporre anche sommariamente questi risultati; diremo piuttosto che le ricerche sperimentali affidate alle varie cattedre e laboratori dell'Istituto (trasformato nel 1924 in R. Istituto Superiore Agrario e Forestale) sono così ripartite:

(1) Economia, statistica e legislazione forestale. (2) Botanica e fitogeografia forestale. (3) Chimica e tecnologia forestale. (4) Geologia e pedologia forestale. (5) Dendrometria e assestamento. (6) Biologia e patologia forestale. (7) Selvicoltura.

Per intensificare ancora maggiormente l'attività sperimentale dell'Istituto e col preciso fine di "Provvedere alla migliore conoscenza e all'incremento della produzione forestale italiana" veniva annessa all'Istituto con legge 3 aprile 1922 la *Stazione Sperimentale di Selvicoltura*. É bene dunque chiarire che la Stazione non è che un organo dell'Istituto e che limita le sue ricerche alla selvicoltura propriamente detta, perchè tutti gli altri rami di ricerche nel campo forestale sono affidati ai singoli laboratori dell'Istituto, come più sopra si è detto. Occorreva però determinare il coordinamento della nuova istituzione coi laboratori stessi; e lo scopo fu ottenuto affidando alla Stazione il particolare compito delle indagini e sperimentazioni in foresta, secondo i programmi di lavoro coordinati con quelli dei laboratori specializzati.

Quanto al programma di lavoro, esso fu stabilito in pieno accordo con la Direzione Generale delle Foreste e col Consiglio Accademico dell'Istituto, affinchè la Stazione fosse chiamata senz'altro a studiare quei problemi che maggiormente interessavano l'Amministrazione forestale dello Stato ed il Paese.

La scelta del programma avvenne in base ad un esame della situazione che qui brevemente riassumiamo.

(1) L'Italia è un paese per 4/5 montuoso; per vicende storiche e demografiche, grandi superfici delle sue montagne sono state spogliate del benefico manto delle primitive foreste, senza poi essere sottoposte a razionale coltura. Esse sono perciò oggi in tristi condizioni, sia per la loro improduttività sia per i danni che arrecano al regime idrogeologico. Per la sistemazione del regime fluviale, per la correzione dei torrenti devastatori e per lo sfruttamento delle energie idrauliche è indispensabile una vasta opera di rimboschimento di queste montagne. La

restaurazione della montagna è problema d'interesse altamente nazionale essendo connessa a tutto il grandioso programma di bonifica, di intensivozione dell'Agricoltura e di incremento delle forze idroelettriche, che oggi viene attuato con nuovo e vigoroso impulso dal Governo fascista.

Le condizioni in cui i rimboschimenti devono essere effettuati, sono però molto difficili, trattandosi di vincere terreni sterili e forti avversità del clima; inoltre la variabilità delle condizioni stesse è estrema, date le enormi differenze dell'ambiente fisico dalle Alpi alla Sicilia. La tecnica dei rimboschimenti deve adattarsi a queste fortissime diversità di condizioni ambientali da luogo a luogo, e perciò essa deve essere basata su di uno studio esatto e sperimentale dell'ambiente fisico, della biologia, dei caratteri colturali, nonchè dei pregi tecnologici delle specie che debbono essere impiegate nei rimboschimenti. E quindi è naturale che uno dei compiti più importanti ed urgenti della sperimentazione forestale sia quello dello studio dei rimboschimenti.

(2) Ma non si tratta solo di accrescere, coi rimboschimenti dei terreni nudi, il patrimonio forestale italiano; si tratta anche di aumentare la produzione dei boschi esistenti. A questo riguardo si fa notare che la produzione attuale è molto scarsa come legname da opera e sopratutto come legname di conifere. L'importazione del legname di conifere e di pasta di cellulosa ottenuta da conifere aumenta sempre più; nel biennio 1924–25 si importarono annualmente in media mc. 3,200,000 di legname di conifere e mc. 700,000 di legname di latifoglie (calcolati allo stato tondo).

Si devono quindi studiare i procedimenti tecnici atti ad elevare la produzione del legname da opera e sopratutto di quello di conifere.

Tale scopo di può raggiungere: (a) con l'aumento di produzione delle fustaie; (b) con la trasformazione dei cedui in fustaie; in particolar modo introducendo nei cedui le specie conifere (operazione detta in francese enrésinement). Ciò, ben s'intende, oltre a dare alle conifere un largo posto nel ribmoschimento dei terreni nudi.

(3) Per la risoluzione di ambedue i suddetti problemi: rimboschimenti e miglioramento dei boschi esistenti, si tratta di operare in condizioni oltremodo vario e perciò è importante profittare non solo delle specie indigene ma anche di quelle esotiche, considerando in special modo che la flora indigena è molto scarsa di conifere proprie delle zone temperate e calde e che, nelle zone caldo-aride, la flora medesima è poverissima di specie atte a fornire legname da opera. L'introduzione di specie forestali esotiche è considerata come un altro importantissimo oggetto della sperimentazione.

Vediamo ora come ha impostato lo studio di questi tre problemi la Stazione Sperimentale di Selvicoltura.

Non potendo, per la iniziale scarsità di mezzi e di personale, affrontare contemporaneamente tutti i problemi, la Stazione sperimentale ha deciso di rimandare ad una seconda fase lo studio del trattamento dei boschi, in vista dell'aumento della loro produzione, tanto più che in questo campo si sta già compiendo un notevole lavoro da parte del Laboratorio di Dendrometria e Assestamento. La Stazione sperimentale ha invece subito iniziato lo studio dei

rimboschimenti e quello della conversione dei boschi cedui, connettendo a questi due la sperimentazione di specie forestali esotiche.

Per lo studio dei rimboschimenti, seguendo il principio esposto dianzi, si è pensato che una serie di indagini molto accurate sui risultati già conseguiti in circa 50 anni di azione in ogni parte d'Italia, avrebbe portato resultati più pronti e sicuri della sperimentazione propriamente dette e che questa avrebbe dovuta essere riservata ad alcuni speciali casi. Essendosi poi riconosciuto che la base scientifica della tecnica del rimboschimento è l'ecologia forestale, si è iniziato lo studio metodico dei climi e dei terreni. Per lo studio del clima la Stazione sperimentale si basa sugli osservatori istituti dallo Ufficio centrale di meteorologia ed al servizio Idrografico del Genio Civile, oltre che su numerosi osservatori impiantati appositamente dalla Direzione Generale della Foresta nelle regioni di montagna; questi ultimi osservatori (attualmente 139) fanno capo alla Stazione Sperimentale di Selvicoltura.

Quanto allo studio dei terreni, esso viene affettuato in collaborazione col Laboratorio di geologia dell'Istituto.

Per la sperimentazione di specie forestali esotiche si è organizzato anzitutto una larga provvista di semi sia con acquisti diretti, sia con la raccolta in Italia e si sono posti in efficienza i vivai forestali annessi alla Stazione a Firenze ed a Vallombrosa.

Si distribuiscono poi largamente i semi nei vivai forestali di tutta Italia dipendenti dall'Amministrazione forestale, e col concorso dei funzionari dell'Amministrazione stessa, si vanno estendendo ad un capo all'altro del Paese le parcelle sperimentali in foresta, che oggi sorpassano il numero di 150. Parte di queste parcelle riguardano il rimboschimento di terreni nudi, parte la sostituzione di specie nei boschi preesistenti, parte la conversione dei cedui.

Un'importanza speciale rivestono le esperienze con numerose specie di *Eucalyptus* ed *Acacia* di provenienza australiana nell'Italia del Sud e delle Isole, allo scopo di studiare l'arricchimento di quelle regioni con specie a rapido accrescimento per la produzione di legname da opera e di corteccie tanniche di alto pregio.

Il lavoro sperimentale per lo studio dei problemi sopra indicati è iniziato da poco più di tre anni, però esso già promette di dare resultati molto importanti ed assumerà certamente maggiore efficacia con l'aumento dei mezzi e del personale addetto alla sperimentazione, al che il Governo ha già provveduto.

An abstract of this paper, in English, was read by the Secretary. Brief comments thereon were made by Prof. N. C. Brown of Syracuse, Prof. J. W. Toumey of Yale and Mr. E. N. Munns of the U. S. Forest Service.

The abstract follows:

ABSTRACT[3]

SERPIERI, A. and A. PAVARI. Forest experimentation as a scientific basis of silviculture, with especial reference to Italy.

[3] In place of the full paper, the accompanying abstract was read at the Congress, and this abstract is included by request. EDITOR.

In this note we do not intend to demonstrate the necessity of forest experimentation as a scientific basis of forestry, this being already universally known; we intend rather to indicate the way in which this experimentation must be understood and organized.

Our study starts on general considerations and comes to particulars regarding the forest experimentation in Italy.

As general principles we establish the following:

(1) The forest experimentation must obey the principle of continuity across very long periods.

(2) The properly conceived experimentation must be integrated by systematic observations on the life and production of the woods.

(3) The observations and the experimental researches must be multiples, that is, executed in the largest possible number of localities, in order to include the variety of the ecological and biological conditions offered in each country. The experiments localized in a few sites have no practical value except for the sites themselves, and cannot be generalized.

(4) Since the period of forest experimentation is very long, it is necessary to organize simultaneously and in the shortest possible time the experiments and the observations regarding a determinate object.

(5) The extent and the simultaneity of the experiments and observations can be realized only when in the stations there are large means and personnel; all to realize the two stated and very important conditions it is advisable also to sacrifice the particularities of the researches.

(6) A strict connection between the experimental institutes and the forest service of the government is necessary to put the experimenters in contact with the necessity of the forest practice.

Forest experimentation in Italy is assigned to the R. Istituto Superiore Agrario e Forestale, Florence. It possesses complete laboratories for the investigation of economy, statistics and forest legislation, botany and phytogeography, chemistry, forest technology, geology and forest pedology dendrometry and management, forest biology and pathology, and silviculture.

In order to intensify the activity of this institute, there was annexed in 1922 the experimental station of silviculture that dedicates its principal activity to the experimental investigation in the forest, connecting this work with the researches carried out of the specialized laboratories.

The experimental Station began its activity in the following directions:

(1) Technics of forestation;

(2) Transformation of the coppices of high forests;

(3) Introduction of forest exotic species.

The work of the experimental station is in strict connection with the functionaries of the forest administration of the state and is extended to the entire country.

Actually the number of experimental areas is more than 150.

SOME ASPECTS OF FOREST INVESTIGATIVE WORK IN CANADA[1]

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The forests of Canada that clothed the shores of the streams and encumbered the portages have always engaged the attention of explorers. The French made quaint reference to their forbidding and somber aspect as they observed them along the great fluviatal highways of the St. Lawrence, and the more observing described the trees and gave them names according to the actual or fancied resemblance to those of the homeland. Like the course of empire, knowledge of the Canadian forests has proceeded westward and—shall I say—northward. More than 3 centuries have passed and there is still abundant opportunity for the exercise of the exploring type of mind, and the mental stimulus of discovery of new facts in regard to the forests is as fresh and keen as at the time the earliest explorers first plunged into the sylvan wilderness. Points of view, however, have changed and opportunities for the application of knowledge have greatly multiplied, so that today the explorer has the added satisfaction of feeling that his discoveries may be of service to the state, not so much in unthinking exploitation and the conversion of wood into the coin of the realm, as in perpetuating the productiveness of its forest resources and thus in maintaining the economic prosperity of his country.

With your indulgence, I propose this morning to outline some of the methods of gaining knowledge of Canadian forest conditions by explorers, past and present, and finally I hope to leave with you some conception of what the more important problems in forest investigation are.

As in the United States, the earliest notable scientific contributions were made by members of the Geological Survey. As early as 1873, Robert Bell, later the Director of the Geological Survey, prepared a map showing the northern limits of tree distribution in the 4 original provinces of the Dominion, and for the next 20 years or more, he published frequent papers upon the character and the distribution of the forests. His work was not all descriptive by any means. For his time and training, he had a remarkably keen insight into the causes of forest composition and distribution. Bell's map of tree distribution has been revised from time to time and is still issued by the Dominion Forest Service at Ottawa.

John Macoun was attached to the Geological Survey as botanist and in that capacity he prepared a "Catalogue of Canadian Plants." The publication of

[1] Presented before the International Cogress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 17, 1926.

this series began in 1884 and continued until 1896; most of the trees are included in Part III, issued in 1886. We owe a great deal to Macoun for establishing the range of species, especially their occurrence in out-of-way and unexpected places. While speaking of the work upon trees as individuals, I may add that the Dominion Forest Service published a descriptive text upon Canadian trees in 1917 under the editorship of R. B. Morton, and, that during the present year, the Provincial Forestry Branch of Ontario has issued a bulletin of remarkable excellence on the trees of that province prepared by J. H. White of the Faculty of Forestry at the University of Toronto.

Reverting for a moment to the work of Bell and Macoun, I would like to say that the most vivid descriptions of destruction by forest fires and the most eloquent appeals for better protection in Canadian forest literature are to be found in their reports. Between 40 and 50 years ago, however, theirs were as voices crying out in the wilderness, a wilderness of public apathy and ignorance of the actual and potential value of the forests; a wilderness, which, although much improved since their day, too much envelopes the Canadian public and too much hampers the real forestry activities of the profession.

The first general classification of the forests of the whole country, by Sargent, is to be found in the "Tenth Census Report of the United States." His report on forest conditions, as you will recall, covered the continent. C. Hart Merriam's report on "Life Zones and Crop Zones" also included Canada in some of its later editions. A more detailed classification than either of these was made by John W. Harshberger in connection with his volume on the plant geography of North America in the Engler and Drude, "Vegetation der Erde" series.

The first, and thus far only, forester to classify the Canadian forests as a whole was the late B. E. Fernow, whose memory the foresters of 2 countries revere and love. He had seen most of the commercial forest areas of Canada and, having a remarkable aptitude for keen and accurate generalization, he produced a classification which the results of nearly 20 years of exploration have only proven to be well founded. A thorough study of the Canadian climate, especially the temperature and rainfall relations of the growing months, preceded the making of the forest map. Although not definitely so stated in the text, his 13 forest regions are, in essential aspects, climatic regions. ["Forestry Quarterly" 6 (No. 4): December, 1908; also "Proceedings" Society American Foresters 7 (No. 2): November, 1912.]

The Natural Resources Branch at Ottawa has recently made a revision of Dr. Fernow's forest map.

Around 1900, the Dominion Forest Service began a series of exploration and reconnaissance surveys on the Dominion lands in the western provinces and these were continued for 15 years. Although not yet complete, they have not been made since the Great War. The primary objects of the work were to demark agricultural and non-agricultural land in advance of settlement and to obtain data for the establishment of forest reserves. The forests were classified and the timber was estimated on the basis of cover types and age class types. The latter task was not difficult because much of the greater portion of the areas contained

stands that had arisen as the result of periodic fires during the past century. The control of the work was obtained through the already established base lines of the land division system and, where these did not exist, through such natural topographic units as river valleys, mountain ranges, and plateaus. As a rule, the boundaries of a forest type were determined by pacing or by the judgment of the eye from some point of advantage. This was, of course, a "rough and ready" method, but it served the purposes of the time. Trained observers, however, really became quite expert in the work. The Dominion Forest Service Bulletins, Numbers 5, 6, 17, 18, 29, 32, 45, and 53, contain the results of such reconnaissance surveys.

More accurate and detailed surveys than those described above were made in Nova Scotia in 1908 and 1909 by the late Dr. B. E. Fernow and the results were published by the Commission of Conservation in 1910 under the title of "Forest Conditions of Nova Scotia." The land survey maps furnished by the provincial government were carried by the investigators in the field, and on these maps there were indicated the forest conditions on each lot of 100 acres, determined either by personal investigation or by reliable information from the owner. Later the facts thus obtained were combined into a forest type map on a scale of 4 miles to 1 inch. The types were classified as coniferous forest, hardwood forest, mixed coniferous-hardwood forest, fire barrens, and natural barrens. The forests were also described on the basis of underlying rock and soil conditions. The condition of the forests with reference to lumbering was indicated by such terms as virgin; lightly, moderately, and severely culled.

As chairman of the Forestry Committee of the Commission of Conservation, Fernow initiated forest surveys in British Columbia, Ontario, and Saskatchewan. The field work and the compilation of the data in British Columbia occupied the time of 2 foresters, Craig and Whitford, for 5 years. Naturally, owing to financial considerations, the size of the province, and the stage of its industrial development, the field work of the survey was not carried on in such detail as that in Nova Scotia, but on the other hand more consideration was given to the business aspects of forestry, lumbering, and the market problems of the province. The Provincial Forestry Branch placed all its data with regard to the forest conditions at the disposal of the investigators. The private lumber companies also contributed. The information thus obtained was generalized in the final report.

The forest types were classified on the basis of composition, climatic, and soil conditions, and physiographic units. In addition, each commercial species was treated separately as to distribution, proportion of occurrence in the various types, silvicultural characteristics, rate of growth, and yield of marketable products.

The results of this survey were published by the Commission of Conservation in 1918 under the title of the "Forests of British Columbia." The volume contains 400 pages and is accompanied by 20 maps showing the distribution of the various types and individual species. This report is the most complete yet published for any province of the Dominion.

The results of the forest survey of Saskatchewan were never published. Two years' work had been done on the survey of Ontario, when the Commission of Conservation was abolished, in 1921.

The Commission of Conservation in 1912 made a detailed survey of 1,300,000 acres in Central Ontario. Like the work in Nova Scotia, the land division maps, having a scale of ¼ mile to 1 inch, were used in the field and the forest conditions on each lot of 100 acres were plotted upon them. The data were finally transferred to a map having a scale of 2 miles to 1 inch. The forests were described by cover types and the stands on the old burns, which constituted a large portion of the area, were recorded by age classes in decades. The economic conditions of the region other than those of the forests were also studied and reported. The results of this work were published by the Commission of Conservation under the title of the "Trent Watershed Survey, Ottawa, 1913."

In 1913 a detailed survey was made of 2 much burned townships, comprising about 80,000 acres within the region included in the survey of 1912. Compass and chain lines were run at intervals of ¼ mile across each township and the trees were calipered down to ½ inch in diameter on a strip 33 feet wide. The primary object of this survey was to determine the effect of repeated fires upon the regeneration of pine. The results were published in Forest Protection in Canada 1913–1914, under the title of the "Effect of Repeated Forest Fires in Methuen and Burleigh Townships, Peterborough County, Ontario, Commission of Conservation, Ottawa, 1915."

In 1920, the Ontario Forestry Branch began a survey of the provincial forest resources, which it is planned to continue until the entire forest area of the province is covered. The work is done for the most part during the summer months by the aid of forestry students under the direction of foresters. Thus far around 40,000,000 acres have been classified as to forest types and the timber and pulpwood thereon have been estimated. The methods of the survey vary according to conditions. Where organized townships exist, the subdivision of the 100-acre lot was used as the unit of classification. Where townships had been blocked out, but not subdivided, the boundary lines were used as base lines from which ordinary cruising strips were run at intervals of ½ mile. The final report in each case was on the basis of the township and the final maps were prepared on the scale of 4 miles to 1 inch. Where there had been no township surveys, as in the greater portion of the Hudson Bay drainage area, the forest conditions were described on the basis of drainage systems. In the more northern regions, where the forest types are simple in composition and usually even-aged, the width of the cruising strip on which the trees were calipered was reduced to 11 feet.

The forests were classified as pure hardwoods, pure conifers (where at least 80 per cent of the stand was of the character indicated), mixed hardwoods and conifers. Each of these was subdivided into virgin, moderately culled, severely culled, second growth, and young growth, young growth being saplings up to 4 inches and second growth being pole wood above 4 inches in diameter. The old burns, covered with poplar and birch, always comprising a considerable

portion of the area, were classified separately as to the size of the trees and the condition of the coniferous regeneration with reference to presence in actual or potential commercial quantities. The trees in each class were listed as standards, poles, and saplings, and the areas occupied by each were recorded and mapped in the final report. This was in reality a rough method of recording the age of the burns.

Photographing forest types from the air has been successfully undertaken for several years in Canada, the pioneer in the work being the Laurentide Air Service under the direction of Ellwood Wilson. The Ontario Forestry Branch, however, has developed air sketching to a remarkable degree of efficiency. This work was inaugurated in Ontario in the summer of 1920, when some 10,000 square miles were mapped from the air. The only maps of the region available were on the scale of 8 miles to 1 inch. These were enlarged to a scale of 1 mile to 1 inch, attached to a drawing board and used for sketching by the observer in the aircraft. The boundaries of the types were sketched in and oriented by means of the known positions of lakes and streams. The composition types were subdivided into such designated size classes as mature stands, at least 75 per cent of the trees of commercial size, polewood stands, not over 25 per cent of commercial size, sapling growth, burned areas naturally restocking, natural and fire-made barrens. This was worked out by landings to relate the appearance from the air to the actual condition of the stand as determined by ground work. Wherever possible during such landings sample plots of types were laid out and measurements recorded. The results of this method were very gratifying, for there was no case in the first season of operation where the aërial classification differed materially from conditions actually found on the ground.

In the following season a combination aërial and ground survey was made of some 8,600,000 acres in the James Bay region. The type maps were prepared by the aerial observer, sketching directly on base maps to a scale of 2 miles to 1 inch. Information from these maps prepared in flight was transferred to other maps which were furnished the ground parties who ran cruising strips through the areas bearing commercial timber. The cruising parties were carried to the more inaccessible places by aircraft, landed with instruments and provisions, and picked up by the planes a week or several weeks later, when their work was finished. For the details of this survey and the forest type map you are referred to the "Report of the James Bay Forest Survey, 1923."

If you wish a more complete discussion of the methods, results, cost, and type of machines used in aërial forest mapping in Canada, you would do well to consult the annual reports of the Minister of Lands and Forests of the Province of Ontario for the years 1921, 1922, 1923, and 1924, the reports of the Dominion Department of National Defense on Civil Aviation, and articles in the "Illustrated Canadian Forest" and "Outdoors" for January, 1920; February, 1922; May, 1922; by Ellwood Wilson, and July, 1924, by F. T. Jenkins.

For the past 10 or 15 years the Forest Service of Quebec has been carrying on a forest and soil classification of townships in advance of settlement or lumbering operations. This work has progressed at the rate of about 10 townships,

some 225,000 acres, a year. The forests are classified into cover types and their content in terms of commercial timber is estimated under the ordinary cruising methods. The soils are classified as sands, loams, and clays, agricultural and non-agricultural. The data are transferred to maps and they are deposited in the head office in Quebec City. Apparently no results of these surveys have been published.

Since 1922 the timber and pulpwood operators on Crown Lands in Quebec have been required to make an inventory of their holdings in order to obtain certain concessions on diameter limit with regard to the cutting regulations. This has led to an intensive classification of the forest lands in that province on the bases of types, yields, and growing stock, involving growth studies and a classification of the natural regeneration. Thus far no results of such surveys have been published.

About 10 years ago the Province of New Brunswick began an intensive survey of its Crown Lands forests. The object was not only to obtain an estimate of the timber at present standing, but also to determine its rate of growth and yield as a basis for regulated cutting operations. Base lines were established by land surveyors and compass and chain lines were run from them at intervals of ¼ mile. The trees were calipered down to 4 inches in diameter on strips 33 feet wide. The forests were classified into composition types, and their yield, in terms of board feed and cords of pulpwood, was estimated. Forest regeneration surveys, as described later, were made in cooperation with the Commission of Conservation. Growth and yield studies were made by the sample plot method. The diameter increment of a large number of trees was determined by Pressler borings. Data with regard to soil conditions, drainage systems, and topography, were also included in the survey. All this information was transferred to maps to the scale of 2 miles to 1 inch. This is the most detailed survey that has been attempted by any province of the Dominion. Since the War, however, owing to financial reasons, the work has been somewhat restricted. Up to 1924, 4,500,000 acres had been classified, which is about 50 per cent of the Crown Land area of the Province. Progress reports of this survey may be found in the annual reports of the Crown Lands, Department of New Brunswick, Fredericton, since 1916.

In 1916, the Commission of Conservation began an investigation of conditions on cut-over, unburned pulpwood lands in eastern Canada, especially in the provinces of New Brunswick, Ontario, and Quebec, with reference to the regeneration of the commercial species. The work was conducted like an ordinary timber cruise under the strip system. Chain and compass lines were run from established base lines at intervals of 20 chains (¼ mile) and all the trees were calipered down to ½ inch in diameter on a strip 33 feet wide. At the end of each fifth chain length the seedlings (trees less than ½ inch in diameter) on a plot a square rod in extent were counted and recorded. In some places wooden posts were placed at intervals of 5 chains, so that the lines can be run again and the conditions periodically recorded. This study was continued for 3 years, when a 5 per cent cruise was made of approximately 150,000 acres of cut-over pulpwood

lands. The results will soon be published as a bulletin of the Dominion Forestry Branch at Ottawa.

As an outgrowth of the regeneration survey work, the Commission of Conservation, in cooperation with the Laurentide Company and the Riordan Company, established a series of permanent sample plots in Quebec on areas of different densities and different ages of natural regeneration. The chief objects of the establishment of the plots were to determine the rate of growth, the rate and cause of the mortality as they advanced in age. The Forest Investigation Section of the Dominion Bureau of Entomology cooperated in the study, and, while the Commission of Conservation existed, mycological studies were made upon the plots.

The plots were ½ an acre or an acre in extent and they were set off with a staffhead compass. The trees were numbered, measured, described as to health, crown class, and the positions plotted on a map. At first, white paint was used in numbering the trees, but, as it proved unsatisfactory, aluminum tags, inserted at the breast height point with brass pins, were finally used. A steel tape proved more accurate than calipers for measuring the diameters.

Sub-plots of a square yard or a square rod were established for the study of seedling and small sapling stands. Much attention was given to seed bed and germination conditions. Seed spots were made in various conditions of ground vegetation, litter, and mineral soil. It was found best to plant a given number of seeds rather than to depend upon irregularities of natural seed fall.

As a further development of the forest regeneration studies on the part of the Commission of Conservation, experimental cutting areas were established in cooperation with the Laurentide Company in Quebec and the Bathurst Company in New Brunswick. The first contained 120 and the second, 500 acres. The chief object was to determine conditions of regeneration following regulated logging operations. A portion of the areas was cut clean in strips oblique to the prevailing wind direction. The strips were from 2 to 4 chains apart and were 1 to 4 chains wide. The débris from the logging operations was left in its natural condition on one strip; it was cut and scattered on the ground in the next; it was piled and burned on the third strip, and on the fourth clean-cut strip the series began again. The other portion of the areas was cut clean in circular patches ½ acre to 1 acre in extent. The débris on these patches was treated as on the clean-cut strips.

With the abolishment of the Commission of Conservation in 1921, the Dominion Forest Service took over the experimental areas. The cooperative work has been extended to the Price Brothers Company of Quebec and the Pejepscot Company in New Brunswick. The Dominion Forest Service has improved the work begun by the Commission of Conservation and has standardized the methods employed on the various areas. At the present time the Dominion Forest Service has under its control about 200 acres of permanent sample plots and 1200 acres of experimental cuttings in eastern Canada.

The largest and most intensive experimental area in Canada is the Petawawa Forest Experiment Station established in 1918 in cooperation with the Depart-

ment of National Defense by the Dominion Forest Service. This is a military reservation on which around 100 square miles have been granted to the Dominion Forest Service for purposes of protection, administration, and experimentation. The area has been very largely cut over and burned over in the past 70 years and is, therefore, chiefly covered with immature stands; only about 10 square miles supporting mature, or over-mature, stands. About 30 per cent of the area is non-forested, consisting of waste places, water, muskeg, and abandoned farms. The area supports representatives of practically all of the forest types to be found in the St. Lawrence drainage basin and is thus admirably adapted to experimental work.

A working plan survey is being made of the reserve with the intention of regulating the management on a sustained yield basis. A secondary working plan is in course of preparation on certain areas of over-mature hardwoods for the purpose of woodlot management. Up to the present time 5 timber sales, in 2 forest types, have been made on the reservation; one type being mature poplar with an under-story of balsam, white pine, and spruce; the other being pure jack pine with abundant white pine regeneration beneath it. In the poplar cuttings, the slash was lopped and scattered; in the jack pine cuttings the slash was burned. Permanent sample plots have been and are being established on these cut-over areas in order accurately to determine just what will happen to the advanced growth and to ascertain the degree and nature of subsequent natural regeneration.

Perhaps the most important work on the Petawawa Forest Experiment Station is in connection with the permanent sample plots, more than 100 of which have been established since 1918. Especially to be mentioned are those dealing with type conversion from poplar or birch stands to coniferous stands and from intolerant conifers to tolerant or partially tolerant conifers; more particularly the effect of different degrees of crown density upon the development and rate of growth of the under-story of small conifers. These old burn types are the most prevalent in eastern Canada and from them must come the greater portion of our future supplies of saw logs and pulpwood. Without carefully planned management their yields will be very low.

The Dominion Forest Service also carries on a program of investigation on the forest reserves in the western provinces. This includes the influence of under vegetation and of slash burning upon natural regeneration, growth and yield studies, and the effect of thinnings.

While discussing in particular the investigation work of the Dominion Forest Service, I may say that you will find a detailed statement of its research problems, methods of procedure, and schedules for permanent records in its recently issued "Forest Research Manual."

It would seem that the study of succession should be the next stage in the investigation of natural forest conditions and, indeed, it has already begun through the work of A. B. Connell, who has taken this point of view in unpublished reports on the forests of eastern Saskatchewan and those of the Missinaibie Valley in Ontario. The Forestry Branch of Ontario has begun a

study under the direction of T. W. Dwight of the Faculty of Forestry at the University of Toronto of the mixed poplar-birch-white pine stands on burned areas. While the primary object of the investigation is the more practical determination of yields of commercial materials, the problem is being approached from the standpoint of succession, beginning with young stands and tracing the changes in composition as they advance to maturity. The Forest Branch of British Columbia is conducting a similar study in the hemlock-cedar type and in the Douglas fir type of the coastal forests.

All of the forest services of Canada and many of the private companies employing foresters have carried on volume table studies. The Dominion Forest Service in the past few years has made a particularly detailed study of the subject and the results are given in a report by Major W. G. Wright entitled "Volume Taper Studies." A bulletin by the same author on the "Use of Statistical Methods in Forest Investigative Work" includes the application of such methods to estimating regeneration surveys and sample plots as well as to volume studies. The Provincial Forestry Branch of Ontario has also under way an investigation of the application of statistical methods to timber cruising work.

I would like, now, to make brief mention of experiments in artificial regeneration. Forest planting in Canada goes back 30 years or more and has made rapid strides in the past 5 years. Some of the older plantations are being measured for growth and yield records and some of them are being thinned. All of the governmental forestry organizations and those of private companies are experimenting with mixtures, density, and adaptability to site.

As you all know, regeneration by direct seeding has not, as a whole, been successful on this continent, but a few experiments have been so successful that there is a tendency among foresters to demand a new trial under carefully regulated and improved technique. This tendency has found expression in practically every province of Canada in experiments in seed sowing, chiefly by the seed spot method. The most extensive experiments are being made in New Brunswick by the Dominion Forest Service in cooperation with the Provincial Forestry Branch and the Dominion Advisory Council of Scientific and Industrial Research, the co-operation of the latter consisting chiefly in furnishing the money, at the rate, I believe, of about $5,000 a year. In that province around 1000 acres in experimental plots have already been established, the plots varying in size from a few acres to 175 acres in extent. They are mostly located in old burns from 5 to 20 years old, but some are in cut-over unburned areas.

The seed spot experiments in New Brunswick are only 3 years old and of course it is too early to draw conclusions from them, but the majority of those sowed to white spruce have been a success from the standpoint of germination and the establishment of the seedlings, and the cost is materially less than that of the ordinary method of planting. Permanent sample plots have been established and records of development from each initial condition will be maintained. The Dominion Forest Service has under consideration plans for supplementing the seed spot experiments with concurrently planted seedling plots so that the

two methods of reforestation may develop together under the same conditions. This would result in more direct and satisfactory comparisons.

At present there is comparatively little investigation of site conditions in the forest by means of instruments of precision, although this stage of research is being initiated by the Ontario Forestry Branch through its measurements of temperatures and humidity in relation to the fire hazard. Many of the data obtained from such investigations can be, and doubtless will be eventually, used for purposes of type and site classification and in the study of the fundamental causes of the establishment and development of natural regeneration.

The investigation of weather conditions in relation to the determination and prediction of fire hazards in Canada deserves more than passing mention. Studies of this kind are being carried on in British Columbia and Ontario by their respective forestry organizations and in the Prairie provinces by the Dominion Forest Service, and all of these organizations receive helpful cooperation from the Dominion Meteorological Service. The latter has issued fire-weather forecast bulletins for certain districts. Thus far the Ontario Forestry Branch has made the most detailed analysis of fire-weather hazard on the basis of measurements of temperature, humidity, wind velocity, and evaporation, carried on for the past 4 seasons. Naturally a good deal of the work has traversed ground already covered by the investigations in the western states, but some very distinct and important contributions have been made, especially with reference to criteria for the grouping of periods of extreme hazard, the relative value of inducing factors, the influence of nocturnal humidity, of late spring and early fall frosts upon hazards, and the size of the area from which records need to be taken. The final test of the value of these investigations lies in the fact that their results have been to a limited extent successfully used in forecasting periods of extreme hazards in central Ontario and in placing extra men and fire fighting equipment in such areas before the predicted fires actually occurred.

For the past 9 years the Forestry Branch of Ontario has carried on studies of fungous diseases of forest trees, particularly of balsam, white pine, and spruce, under the direction of J. H. Faull, head of the Department of Botany at the University of Toronto. The Dominion Department of Agriculture has several investigators at work on tree diseases. The Bureau of Entomology maintains a section with several trained men who are studying the insect pests of forest trees, and it supports field stations for this work in British Columbia and New Brunswick. The Bureau has published comprehensive reports upon the more destructive bark beetles and the spruce budworm. The work on forest insects is in charge of J. M. Swaine, who received his doctorate from Cornell University.

As you know, eastern Canada is largely dependent upon the United States for its fuel supply. The result is that around $100,000,000 go to a foreign country each year. The Dominion Fuel Board at Ottawa is endeavoring to devise means of checking some of this drain and to this end is investigating the wood fuel supply in eastern Canada. The work is in charge of W. N. Millar, of the Faculty

of Forestry at the University of Toronto, who has a report ready for publication on the farm woodlot situation in Ontario.

It may seem to you that this paper deals largely with forest description and survey work. This may be true, but I have attempted to present the matter of forest investigative work in Canada from an historical standpoint and in a new country, naturally, the first thing to do is to find out the nature and extent of the forests. The stage following general description is stock taking. Before a forester can plan for the future, he must know how much he had and only very recently have Canadian foresters been able to make a reasonably reliable estimate of the timber and pulpwood supplies of the country. Hence the preponderance of forest description and forest survey literature in Canada.

What may be called research upon the fundamental problems of silviculture is of very recent origin in Canada. I think I am not unjust to anyone when I say that it is not much more than 10 years old. Considering the fact that the greater portion of the forester's energy is expended in fire protection, and considering that in industrial development Canada is in her adolescence, and is just beginning to realize her powers, the progress made in research, while not entirely satisfactory, is at least commendable. We cannot accomplish all that we should in investigative work without more men especially trained in the methods and processes of research. Research cannot be successfully prosecuted as a side issue to administration. As rare as a blue moon is the combination of a good administrator and a good investigator. On the other hand never shall we get very far towards the solution of our problems so long as the sentiment persists which we often hear expressed in these words: "He will never make a good administrative officer, put him into investigative work." While I believe that a good research student, like a poet, is born, not made, like a poet he can not reach the full expression of his particular qualities of mind without a thoroughgoing training in his art. I feel quite strongly that the profession has emphasized too much its research problems in proportion to the necessity of furnishing properly trained men to solve those problems.

As I look upon them our main research problems in Canada may be outlined somewhat as follows:

(1) Investigation into climatic conditions in relation to the determination and prediction of forest fire hazards. I put this first because our fire protection must be materially improved if we are to have in the future highly productive forests. The solution of the problem does not lie entirely along administrative lines. Fire protection is a serious business and like any other business may be improved by the application of the results of research.

(2) A thorough study of forest types from the standpoint of their casual factors, their life history, succession, and conditions that lead to relative stability, to the end that they may be classified in an easily understandable manner. This seems fundamentally necessary before we can make an intelligent application of the results derived from the permanent sample plots and regeneration surveys. As a corollary to this, there is the perplexing problem of site classification for the determination and application of yields.

(3) A study of the natural seed bed conditions of the commercial species. This again is fundamental if we are to employ silvicultural systems to obtain natural regeneration. It can not be left to chance.

(4) Experimental liberation cuttings in the old burn types so that the valuable regeneration of conifers may be made available. Extensive areas in eastern Canada are covered with poplar and birch with sufficient regeneration of conifers beneath to indicate heavy future yields, but if left alone most of it is killed or hopelessly suppressed. It is an open question whether or not this advanced growth may not be made available for future use through liberation cuttings more cheaply than by letting nature take her course and eventually replacing the lost conifers by artificial regeneration.

(5) A careful study of the technique of seed sowing methods. Repeated fires are creating large areas of waste land. They are already so extensive that the chances are they will never be reforested by planting, the cost being prohibitive. It is within the realm of probability, at least, that a commercial forest can be re-established on them by direct seeding.

(6) Growth and yield studies. Pulp and paper companies are granted large areas of forest land on the assumption by the government that they will have continuous supplies of spruce. For the most part, however, this assumption has very little scientific basis. This policy, if continued, may lead to disastrous economic results. It is evident that the future supplies of white pine must come largely from the young stands established on old burned areas. We should make a determined effort to find out what yield may be expected from this second growth pine. The determination of this would influence the fire protection policy as well as the planting policy.

We earnestly hope that the forest investigative work in Canada, which I fear I have outlined to you quite poorly, will lead up to regulated forests. I expect to live to see the day when at least some simple system of silvicultural management will be in operation on the crown land forests of eastern Canada. There is no technical reason why the forests of Canada may not supply the wood-consuming demands of the whole continent, and indeed, those of many lands beyond the seven seas. The consummation of her possibilities in continuous forest production lies in the lap of the gods—and in the brains of her foresters.

INITIAL ROOT HABIT IN AMERICAN TREES AND ITS BEARING ON REGENERATION[1]

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For 20 years the writer has been interested in germination experiments on forest tree seeds and in the investigation of the initial root-habit of tree seedlings. The term, initial root-habit, is employed to designate the form and habit of root that characterizes a particular species from the time the seed germinates until the young seedlings become more or less strikingly modified in root-form and habit by environmental conditions, more particularly soil moisture.

It is well known that when a given lot of seed, such for instance as white pine, is sown, the initial shoots of the seedlings are strikingly alike as to habit under all conditions of environment. This inherent tendency toward uniformity appears to become weaker as the plants become older, and variety results from variation in external conditions. What is true of the parts of the seedlings above ground is equally true of the roots. The root systems of seedlings of a given species are at first strikingly alike as to form and habit under all environmental conditions that permit their growth and development. If 50 species of tree seedlings are sown under uniform conditions of climate and soil in a single seed-bed, each species will not only exhibit a characteristic form of initial shoot but will also exhibit a characteristic form of initial root.

The progressive development in the root systems of nearly 100 species of American trees has been studied when grown under a wide variety of soil conditions from the time of germination until the end of the first growing season. Several thousand individual specimens have been carefully lifted from the soil and measured, and 700 photographs have been made illustrating their typical root forms at various times during the first season's growth. The data assembled show that the initial juvenile root system of each species follows a definite course of development and maintains a characteristic form for a rather definite period of time following germination. This early inherent tendency not to vary or change in form under change in external conditions, varies greatly with the different species. In all cases, however, the tendency to change under change in external conditions becomes more pronounced as the seedlings become older. It appears, therefore, that there is in each species a strong inherent tendency to develop a definite type of initial root. Some species, however, exhibit much earlier tendencies to change under change in external conditions than is the case with others.

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 17, 1926.

The sugar pine (*Pinus lambertiana*) growing in the same seed-bed with the glaucous willow (*Salix discolor*) at New Haven, developed an initial root which attained a depth of 4–6 inches before the cotyledons were free from the seed coats. In 10 weeks after germination the tap root had penetrated to a depth of 12–18 inches. By the end of the first growing season the tap root had reached a depth of 22–30 inches and there were but few weak laterals and these mostly 12 inches or more below the surface (Fig. 1). In the moist, partially shaded seed-bed at New Haven it has essentially the same initial root habit that it has in its native habitat in the mountains of California (Fig. 8 a and b). On the other hand, the willow developed a comparatively short tap root and reached a depth of from 3 to 5 inches the first season. Strong, rapidly growing lateral roots appeared shortly after the cotyledons unfolded which grew out horizontally just beneath the soil surface. At the end of the first season's growth some of these had reached a length of 12–14 inches (Fig. 2).

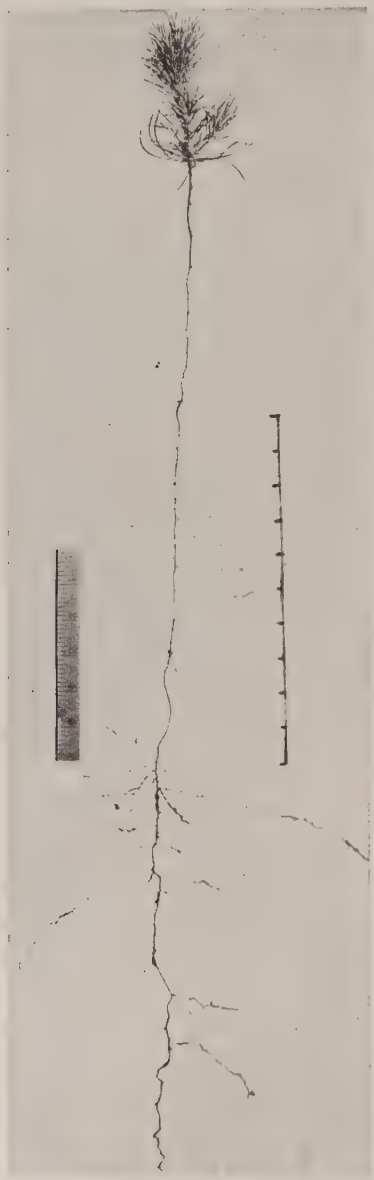

Fig. 1. The sugar pine (*Pinus Lambertiana*) after the first season's growth (October 1) in seed bed at New Haven. Tap root 28 inches long. Origin of longer laterals 12 inches or more below the surface. Typical dry upland type. Inflexible initial root.

The initial root system of the species examined exhibit widely different degrees of flexibility or tendency to change with change in external conditions. Thus the root system of the red maple (*Acer rubrum*) is extremely flexible and begins to show marked modification in form and habit soon after germination, when subjected to different external conditions. It is well known that the red maple grows naturally in swamps as well as on high, dry upland. When the seeds germinate in swamps the tap root remains short (Fig. 3 a) and long laterals spread out beneath the surface. On the other hand when the seeds germinate on upland a deeply penetrating tap root is developed and much shorter laterals (Fig. 3 b). This great flexibility in the initial root habit in red maple or its strong inherent tendency to change with external conditions enables it to germinate and survive over the first growing season on all sites varying from swamp to dry upland.

The bald cypress (*Taxodium distichum*) commonly found intermixed with red maple growing in southern swamps, never follows red maple to dry upland soils. The explanation is found in the high degree of non-flexibility in its initial roct habit. It cannot be forced into developing an essentially deeper root system during its juvenile stage of development by growing on dry upland soil and consequently in such soils it is unable to survive over the first season.

Fig. 2. The glaucous willow (*Salix discolor*) at the end of the first season's growth (October 1) growing in the same seed-bed as the sugar pine shown in Figure 1. Typical swamp type. Inflexible initial root.

The bald cypress developed a short tap root which in seed-beds at New Haven on upland loam never reached a greater depth than from 2–3 inches the first season. As soon as germination took place surface lateral roots with numerous branches were formed which reached a length of 3–6 inches the first season (Fig. 4). The nonflexibility in the initial root-habit of this species inhibits it from becoming established on uplands, although it will grow if planted there when the trees are 2 or more years old and when the root system has become more flexible and better able to adjust itself to change in external conditions. Another swamp species with relatively inflexible initial root habit and never found on upland is the sour tupelo (*Nyssa ogeche*) of southern swamps. This species, although having a more pronounced juvenile tap root than the bald cypress has the same type of initial root habit. The tap root never reached a greater depth of root penetration than 5–7 inches the first season in seed-beds at New Haven. Strong surface laterals begin to develop soon after germination (Pl. I, Fig. 1).

The upland hickories all have a very characteristic initial root habit. The juvenile root system consists of a long rapidly growing tap root with few weak laterals as illustrated in the pecan (*Carya pecan*) (Pl. I, Fig. 2). The pig-nut hickory (*Carya cordiformis*) is a characteristic tree of the dry upland soils of southern New England and it readily establishes itself on open sites when viable seeds are available. The same is true of red cedar (*Juniperus virginiana*) which commonly becomes established on high, dry, pastures. In both of these the initial root habit is such that following germination it rapidly reaches a depth that

Fig. 3 a and b. The red maple (*Acer rubrum*) showing flexible initial root habit. a. Typical form of the root of a seedling after one season's growth in the natural habitat in a swamp near New Haven, Conn. Root penetration 2 3 inches, wide spreading lateral roots. b. Typical root of a seedling after one season's growth in its natural habitat on dry upland near New Haven, Conn. Root penetration 10 inches. Laterals less strongly developed than in Figure 3 a.

permits the seedling to survive during periods of summer drought, when the surface soil is dried out to the point of water non-availability. Moreover, this tendency in the above species and others characteristic of open dry sites to develop rapidly-growing tap roots and few weak laterals results in an initial root habit which is relatively inflexible; it can not be forced to develop strong laterals during the first season's growth even on sites having wet surface soil. In other words, changes in external conditions react on the seedlings of the pig-

nut hickory and the red cedar in a different manner than they do on the seedlings of the red maple which has an initial root habit of great flexibility. In the pig-nut hickory the tap root, following germination, growing rapidly downward, attains a length of 8–10 inches before the shoot is fairly out of the ground. When grown in seed-beds at New Haven, it has attained a soil penetration of some 30–32 inches at the end of the first growing season while the shoot is but 3–5 inches in length (Pl. I, Fig. 5). The long tap root is thick and heavy while the few laterals are extremely small and weak.

If a small stone or other similar obstruction is placed beneath the germinating seed of an upland hickory some 3 inches below the surface the root curves and grows along the horizontal surface of the obstruction for a distance of approximately 3 inches. If the edge of the obstruction is reached within this distance the root again curves and continues its downward penetration in the soil. If, however, the obstruction be sufficiently broad so that the root growing along it for a distance of approximately 3 inches does not reach the edge, it no longer continues to elongate in the same direction but curves in the same plane and forms a spiral (Pl. I, Fig. 3). By stretching out this spiral at the end of the first season's growth it was found that the tap root had approximately the same length as it would have had had it not met the obstruction. Meeting the obstruction and forced to grow in the upper 3 inches of soil did not cause the root to break up into numerous laterals or otherwise change its strong inherent tendency to produce a definite form of initial root. This non-flexible character of the initial root of the pig-nut hickory is shown in its apparent inability to produce strong laterals even when the tap root is removed some 3 inches below the surface. Some 2 weeks after germination the tap roots of a number of seedlings were removed in place. Two months later 3 seedlings had produced no con-

Fig. 4. The bald cypress (*Taxodium distichum*). Typical form of the root of a seedling grown in seed-bed with sugar pine, Figure 1, after one season's growth. Note the comparatively large shoot and small root. Depth of root penetration 2½ inches. Lateral root spread 6 inches. Typical swamp type. Inflexible initial root.

spicuous lateral roots but the portions of the tap roots that remained had greatly enlarged and had taken on the forms of small turnips or radishes (Pl. I, Fig. 4).

In the case of the water hickory (*Carya aquatica*) indigenous to our southern swamps the initial root habit is strikingly different. It has become greatly modified as compared with that of the pig-nut hickory in order to adjust it to a swamp environment. This species, however, like upland hickories shows a high degree of non-flexibility in its initial root habit and is unable to germinate and become established on upland. Seeds sown in New Haven in the same seed-bed with pig-nut hickory produced seedlings with a relatively short tap root but the lateral roots began to develop soon after the seeds germinated. Numerous strong laterals arose later from the base of the shoot at the very surface of the soil (Fig. 5). This early development of a new surface root system through the development of roots from the base of the shoot is undoubtedly an inherent characteristic due to its swamp environment. The tendency to produce these roots, however, persists even when the seedlings are grown in well drained seed- beds.

Fig. 5. The water hickory (*Carya aquatica*) after one season's growth in seed-bed at New Haven, showing a comparatively large shoot and small root. Lateral surface roots arising at the base of the shoot. Typical swamp type. Inflexible initial root habit.

The yellow birch (*Betula lutea*) is a characteristic swamp species except in alpine regions. Only in the northern part of its range is it a conspicuous element in upland forests. Here the surface soil remains moist throughout the growing season. This species is extremely exacting as to soil moisture and never becomes established on dry sites. On the other hand grey birch (*Betula populifolia*) is found on the poorest, dryest soils, and natural reproduction is abundant on open sites where few other species are able to become established. This species is also found in swamps. The investigation of the initial root habit of the yellow birch reveals its strong inherent tendency to non-variability under external influences while a high degree of variability characterizes the initial root habit of the grey birch. The yellow birch on germination produces an extremely short, slow growing tap root which seldom attains a greater penetration than 2–4 inches the first season. When but a few days old, strong lateral roots begin to develop which occupy the first 4 inches of surface soil for a period of some months after germination (Fig. 6).

Grey birch growing in the same seed-bed with yellow birch and having a more flexible initial root habit, reaches a soil depth of some 9–12 inches in the same length of time (Fig. 7). The grey birch, however, unlike the red cedar, pig-nut hickory, and other species with rapidly growing single tap roots and weak laterals in their juvenile stage has strongly developed laterals, one or more of which often bent downward and made sufficiently rapid growth to reach a soil depth which permits survival during periods of summer drought on dry upland soil. This explains survival when germination takes place on open sites that are relatively dry.

In the Sierra Mountains of California, which experience a dry season of several months' duration, the surface soil often dries below the point of water availability to a depth of 1 foot or more. As the seeds of the indigenous species germinate, the young seedlings develop rapidly growing, deeply penetrating,

Fig. 6. The yellow birch (*Betula lutea*) at end of first season's growth, grown in same seed-bed with the sugar pine and hickories. Typical form of the root 4 months after germination. Typical swamp or moist surface soil type. Inflexible initial root habit.

initial tap roots, usually with a few weak laterals. This is well illustrated in the sugar pine (Figs. 1 and 8 b). The seedlings of this species produce juvenile root systems which reach a depth of 14–24 inches the first season and are for this reason comparatively safe from destruction due to the desiccation of the surface soil over the long period of summer drought. The seeds of sugar pine when brought to New Haven, Connecticut, and sown in seed-beds which were shaded and watered over the growing season produced seedlings having the same initial root habit as in their natural habitat in the mountains of California. This species and others which have a rapidly growing and deeply penetrating initial tap root and few weak laterals, later developed strong wide spreading laterals but nener until a depth of root penetration was attained that made them relatively secure

against destruction from summer drought in their natural habitat. Thus the sugar pine in the mountains of California develops much stronger laterals during its second year's growth (Fig. 8 a). When 6 years old many of the lateral roots are 2–3 times as long as the tap root (Fig. 8 b).

Although changes in environment, particularly in soil moisture conditions, do not result in changes in root form until a variable length of time after germination, the typical root form in all species appears to be more subject to change under the effect of the environment as the individual plants become older. In species like the red maple, with extremely plastic juvenile root systems, changes in soil moisture conditions induce changes in root form which may be perceived as early as 10 days after germination. On the other hand, in species like the bald cypress and the pig-nut hickory, that have extremely non-plastic initial root systems, changes in soil moisture conditions do not induce perceivable changes in the form of the juvenile root system for a period of several months after germination takes place.

Fig. 7. The grey birch (*Betula populifolia*) grown in same seed-bed with yellow birch (Fig. 6) and of same age. Characteristic of both dry and wet sites. Relatively flexible initial root habit.

It appears that the juvenile root-form of a given species is determined by inherent characteristics; that this form persists under all conditions of soil moisture for a variable length of time varying from a few days following germination to the entire growing season. In other words, the initial root habit of one species as compared with another varies from extremely flexible to extremely non-flexible under different soil moisture conditions, and the different species represent every gradation between the two.

Most of the oaks have root systems much alike in their early development namely, a rapidly growing single tap root with few weak laterals. This type of root persists for several months but varies in different species in the depth of root penetration attained during the first season and in the size and relative vigor of the lateral roots. Thus, in the post oak (*Quercus stellata*) and in the scarlet oak (*Quercus coccinea*) the initial root attains a length of 16–32 inches the first season in seed-beds in New Haven, and the laterals are small and weak (Figs. 8 c and d). In the red oak (*Quercus borealis*) the tap root is much shorter and the laterals

Fig. 8 a and b. The post oak (*Quercus stellata*) and scarlet oak (*Quercus coccinea*) grown in seed-bed at New Haven with yellow birch and bald cypress, Figs. 6 and 4. a. The post oak, showing typical root form at end of first season. b. The scarlet oak showing typical root form at end of first season.

Fig. 8 c and d. The sugar pine (*Pinus Lambertiana*), showing seedlings with the roots exposed, growing in its natural habitat in the mountains of California. a. A 2-year-old seedling showing the earlier state in the development of the long lateral roots. b. A 6-year-old seedling showing the lateral roots 2 or more times the length of the tap root.

are much heavier and longer. Due to the greater response in the initial root system of the red oak to change, with change in external conditions the seedlings of this species exhibit a relatively wide range in root form at the end of the first season's growth (Pl. II, Figs. 1 and 2). The initial root habit of the post oak and the scarlet oak appears to be much less flexible than in the red oak. If an obstruction is placed in the soil 3 inches beneath germinating red oak acorns, when the root tips reach the obstruction their growth is arrested, numerous laterals are thrown out and the root system becomes diversified (Pl. II, Fig. 2). On the other hand, when the roots of the post oak and white oak meet obstructions in the soil they behave more like the upland hickories. They are much less flexible.

Fig. 9. Vertical excavation in a seed-bed at New Haven, Connecticut, showing a 5-inch layer of loam over a 4-inch layer of crushed rock.

A seed-bed constructed at New Haven, by introducing a layer of finely crushed rock 4 inches in thickness, 5 inches beneath a surface layer of sandy loam, was sown with 42 species of hardwoods and conifers (Fig. 9). The chestnut (*Castanea dentata*), white oak (*Quercus alba*), post oak (*Quercus stellata*), sugar pine (*Pinus Lambertiana*), single leaf nut pine (*Pinus monophylla*), and red cedar (*Juniperus virginiana*), which have initial root systems with a high degree of non-plasticity, developed tap roots which grew down through the layer of

crushed rock. There was no appreciable change in the lateral roots with the exception of their almost complete absence in the layer of crushed rock (Pl. II, Figs. 3 and 4). In the red oak, on the other hand, the downward growth of the tap root was arrested when it reached the rock layer, and only 9 per cent had penetrated this layer at the end of the first season. Numerous strong lateral roots had developed in the layer of loam above the layer of crushed rock (Pl. III, Fig. 1). The tap root of the sugar pine readily penetrated the layer of crushed rock with no material change in the size and general character of the lateral roots, thus showing a high degree of non-flexibility. The root system of the incense cedar (*Libocedrus decurrens*) appeared to be much more flexible and the tap root seldom penetrated the layer of crushed rock. Numerous strong laterals, however, developed in the layer of soil above the crushed rock.

These illustrations which have been selected from more than 100 that might be taken from material examined indicate:

1. The initial root system in form and habit of growth is characteristic of each species, and is closely correlated with the particular site conditions under which the species naturally grows. Swamp species with non-flexible root habit, such as the bald cypress and yellow birch, do not occur under a wide range of surface soil moisture conditions. The bald cypress is never found on upland except when planted. It germinates and becomes established in small openings in swamps where the surface soil is continually moist and where a root penetration of 2 to 4 inches will safely carry it through the first season. The yellow birch, also a swamp species but more tolerant, not only grows in swamps but it is a common upland tree throughout the northern portions of its range where it germinates on decayed logs and in moist duff which does not become overdry during the summer. Although the initial root system remains characteristically superficial over the first season and has a high degree of non-flexibility it is able to obtain sufficient moisture to survive on these upland sites due to the effect of the canopy and duff on surface soil moisture. On the other hand, red maple, one of the commonest species on wet sites is also found on extremely dry sites. It becomes established on sites exhibiting wide variation in surface soil moisture conditions, due to great flexibility in its initial root habit or its inherent tendency to change its early root-form with change in soil moisture conditions.

Tolerant species like hemlock and spruce characteristic of upland soils have short, superficial initial root systems that seldom reach a greater depth than 3–5 inches the first season (Pl. III, Figs. 2 and 3). They never germinate and become established on upland in the open. Under natural conditions they become established under the canopy of an overwood where the largest percentage of available moisture remains in the top soil throughout the growing season.

Intolerant species like the red cedar (*Juniperus virginiana*) and grey birch (*Betula populifolia*) which germinate and become established on open, upland soils, subject to the excessive drying of the top layer during periods of summer drought, produce rapidly growing, deep-seated, initial root systems which place them out of danger of destruction by summer drought when the top soil is dried below the point of water availability.

2. The initial root habit of the many species examined varied greatly in their inherent tendency to change under variations in external conditions. The root systems of some species are very plastic, and quickly become adjusted to a particular environment, more especially to the soil moisture conditions where the seed chanced to fall and germinate. The initial root systems of other species are very inflexible and are unable to adjust themselves to radical changes in soil moisture conditions. Between these 2 extremes is every degree of flexibility.

After the root systems of the various trees examined grew beyond the period of relative non-inflexibility in initial root habit, they no longer could be classified into definite types. Their reaction to external conditions gradually became more pronounced and environmental conditions rather than inherent tendencies determine the form of the root-system.

Due to the great variation on different sites in the moisture content of the superficial soil layers during dry weather and the seedling's necessity for compensation between water loss and water supply, the developing root must reach a soil depth where it is able to meet the demands of the particular species to sustain transpiration and growth. In order to do this the initial root habit of the species must be correlated with the tendency in the surface soil of its particular habitat to become dried to the point of water non-availability during periods of drought.

There are but 2 distinct types of initial root habit in the many species examined. These types are based on the rapidity of the downward growth of the tap root following germination and the consequent depth of soil penetration during juvenile growth. One type is the rapidly growing and deeply penetrating juvenile tap root and the other type the slow growing short tap root. In the former type the lateral roots are either strongly developed and wide-spreading as in the tulip (Pl. III. Fig. 4) and in the black walnut (Fig. 10), or weakly developed and short as in the pig-nut hickory (Pl. I, Fig. 5) and in the sugar pine (Fig. 1). In the other type the lateral roots are always strongly developed and wide-spreading as in the yellow birch (Fig. 6) and the bald cypress (Fig. 4). Every variation in depth and rapidity of juvenile root penetration and in the lateral expansion of the juvenile root system is found in the different species depending primarily on the available soil moisture characteristic of the superficial layers of the soil and the moisture requirements of the species. The rapidly growing, deeply penetrating, juvenile tap root with few weak laterals is characteristic of species of arid regions, of localities having extended periods of summer drought and of dry sites in humid regions in species which naturally become established in the open. In general, it is characteristic of all species that naturally germinate and survive the first season in soils where the surface layers are likely to dry out below the point of water availability during the growing season.

The slow growing, short juvenile tap root with wide-spreading laterals is characteristic of swamp species and also of upland species which, like hemlock (*Tsuga canadensis*) and dogwood (*Cornus florida*), germinate and become established under canopies where the top soil at critical periods retains more available moisture than is retained at greater depths.

The rapidly growing, deeply penetrating juvenile tap root with wide-spreading laterals is characteristic of species that naturally occur on relatively deep, well drained, fine textured soils of valleys, coves, and lower slopes, in regions that experience well distributed summer rains. This form of initial root is characteristic of species which germinate and become established on soils which, due to their physical characteristics and chemical organization, and due to the distribution of the precipitation, maintain a fairly uniform available water content to considerable depth. The black walnut (*Juglans nigra*) characteristic of deep loam soils of bottom lands and the tulip (*Liriodendron tulipifera*), a widely distributed species of eastern United States, illustrate this form of initial root habit. In the former species the strong tap root develops rapidly, often reaching a root penetration of some 25–27 inches the first season following germination. In the latter species the tap root often reaches a depth of 12–18 inches in the same length of time. In both species, however, two weeks or less after germination, the uppermost strong lateral roots are nearly as long and in some cases longer than the tap root (Pl. III, Fig. 4; Fig. 10). Seedlings having this form of initial root habit draw their moisture and nutrients very largely from the more fertile surface soil although at times of drought they rely on the deeper layers of soil for survival.

In order that seedlings survive over the first growing season following germination, the downward growth of the root must keep in advance of the over drying of the surface layers of the soil during periods of drought. Thus, hemlock in southern New England often germinates in great abundance on open sites in the spring when the soil is moist. Due, however, to the lack of adjustment in its initial root habit it does not survive the first summer drought. On the other hand, red cedar germinating on the same site and at the same time becomes established, due to its having an entirely different initial root habit. The downward penetration of the root keeps well in advance of the downward desiccation of the surface soil during periods of drought.

The initial root habit of a given species and its inherent capacity for adjustment to variations in soil moisture conditions appear to be the determining factors in survival following germination on any given site. Even the most intolerant species will germinate and survive over the early juvenile stage under the densest forest canopy, provided the roots are in contact with available moisture. When seedlings die under canopies during the first season's growth, it does not appear to be due to insufficient light but rather to the competing vegetation which withdraws the soil moisture below the point of availability during prolonged periods without rain. When a dense stand is opened up by a shelterwood or selection cutting, the reproduction that appears in the openings is not a response to increased light, as generally supposed, but to increased soil moisture. The trees removed in the cutting have relieved the soil at those particular places where the trees grew, of loss of water through transpiration. The root systems of the felled trees no longer functioning do not remove water from the soil. As a consequence the young seedlings that start in the openings are able to survive during critical periods of summer drought.

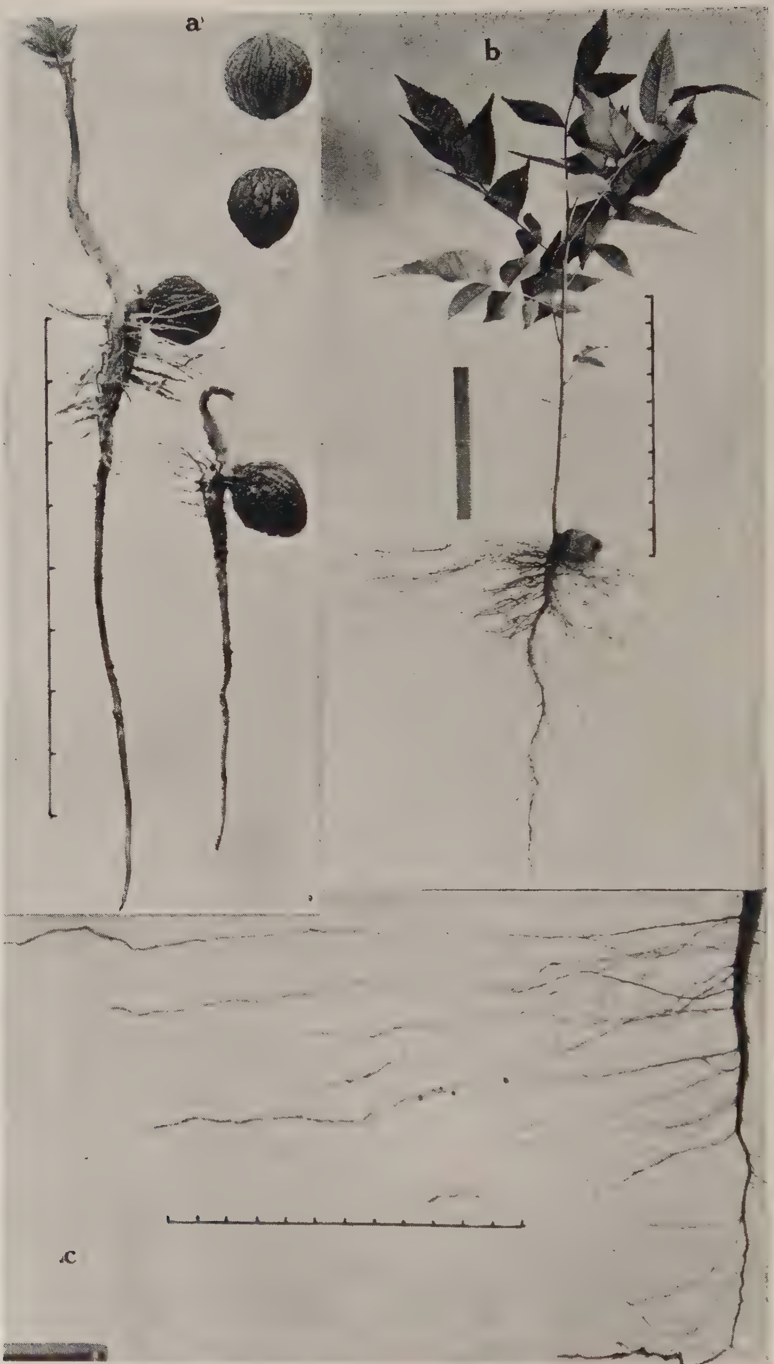

Fig. 10. The black walnut (*Juglans nigra*) grown in seed-bed at New Haven. Same type as in Plate III, Figure 4. a. One to 5 days after germination. b. Six weeks after germination. c. At end of first season's growth.

Since initial root habit is an inherent characteristic and its degree of flexibility or its tendency to become modified under change in external conditions is extremely variable in different species this knowledge is fundamental in explaining natural reproduction and succession on any given site. A study of the initial root habit of our trees and their inherent tendency to react to change in external conditions is also a basis for judging probable survival in various species when seeded on particular sites.

Explanation of Plates

PLATE I

Fig. 1. The sour tupelo (*Nyssa Ogeche*). Typical form of the root of a seedling grown in a seed-bed with sugar pine (Text Fig. 1). After one season's growth. Note the comparatively large shoot and small root. Depth of root penetration 7 inches; spread of lateral roots 7 inches. Typical swamp type. Inflexible initial root.

Fig. 2. A pecan seedling (*Carya pecan*) grown in a seed-bed at New Haven, Connecticut, with the bald cypress and sour tupelo (Text Fig. 4 and Pl. I, Fig. 1). The figure shows seedling at the end of the first season's growth. Note the small shoot and large root. Typical dry upland type. Inflexible initial root. Length of shoot 5 inches. Length of root 28–32 inches. Few weak lateral roots.

Figs. 3 and 4. The pig-nut hickory (*Carya cordiformis*) grown in a seed-bed at New Haven. 3. Seedling showing spiral form of tap root due to obstruction in the soil 3 inches below the surface. 4. Seedling with tap root cut off in place 3 inches below the surface, two weeks after germination. Photographed 6 weeks later.

Fig. 5. The pig-nut hickory (*Carya cordiformis*) grown in a seed-bed at New Haven, Conn., with the bald cypress and sour tupelo (Text Fig. 4. and Pl. I, Fig. 1.). Note the small shoot and long root at end of first season's growth. Root penetration 36 inches. Typical dry upland type. Inflexible initial root habit.

PLATE II

Figs. 1. and 2. The red oak (*Quercus borealis*) grown in a seed-bed at New Haven. 1. Typical form of the root at end of first Season's growth. Note the relatively strong development of the surface lateral roots. 2. Form of the root due to obstruction placed 3 inches below the surface. Seedling 9 weeks old. Note the vigorous development of lateral roots. A moderately flexible initial root habit.

Figs. 3 and 4. The pig-nut hickory (*Carya cordiformis*) and the red cedar (*Juniperus occidentalis* growing in seed-bed with layer of crushed rock 5 inches below the surface. 3. The pig-nut hickory at end of first season's growth. Note the absence of lateral roots where the tap root passed through the layer of crushed rock. 4. The red cedar at end of first season's growth.

PLATE III

Fig. 1. The red oak (*Quercus borealis*) grown in a seed-bed having a layer of crushed rock 5 inches below the surface. Due to the flexibility of the initial root habit the downward growth of the tap root was arrested and strong lateral roots developed. Photographed at end of season's growth.

Figs. 2 and 3. The sitka spruce (*Picea sitchensis*) grown in a seed-bed at New Haven. 2. Typical form of root 2 months after germination. 3. Typical form of root at end of first season's growth.

Fig. 4. The tulip (*Liriodendron tulipifera*) grown in a seed-bed at New Haven. At end of first season's growth, illustrating the initial root form with rapidly growing, deeply penetrating tap root and strongly developed wide-spreading laterals.

Toumey: Root Habit in American Trees

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METHODS AND AIDS IN TREE FORM INVESTIGATIONS AND IN THE CALCULATION OF VOLUME, YIELD OF VARIOUS PRODUCTS, AND GROWTH[1]

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Not only for research work in forest science, but also in instruction, in formulating working plans, in making surveys of the forest resources of an estate or of an entire nation, and not least in the practical business activity of the wood-using industries, a well-worked out system for estimating standing timber is of very great importance. Not rarely, however, the necessary methods of procedure in this work have developed along essentially different lines, partly in different countries, and partly within the same country, according to the purpose to be served, as outlined above. Thus, for instance, it has happened that in the industrial and business world often purely local trade customs and estimation methods have developed by means of which the desired estimates for a certain tract or for the purposes of a certain industry are striven for, yet the results are useless on other tracts or sometimes even on the same tract should marketing conditions for the timber change in the course of time. As an example of such a system I may here only mention the estimation of round timber in board feet by an endless number of log rules, as well as the estimation of forests by volume tables constructed for a certain top diameter, this minimum dimension varying with both place and time. Another frequently noted characteristic of these so-to-speak commercial estimation methods is that they are rather rarely of such accuracy that they are applicable in scientific work.

Scientists have therefore often been compelled to proceed along other lines, commonly based on the determination of the exact volume of wood in the bole, although now and then, as in Germany, limited to a minimum top diameter (Derbholz) determined once and for all. An important motive for the introduction of these exact methods of measurement lies in the yield investigations carried out by different experiment stations, looking toward the elucidation of the course of growth under different conditions. Here the difficulty of obtaining satisfactory results lies precisely in the more or less insufficiency of the practical methods of measurement and calculation.

In this manner scientific work has come to occupy a double position, far from advantageous in that its results, in consequence of the dissimilarity of estimation methods, have not been able to be made use of by practical men, who are seldom

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 19, 1926.

interested in exact yield, but only the yield of timber for industrial uses. To devise a cure for these anomalous conditions through the introduction of a single unified system of mensuration may only be possible in exceptional cases; instead, in most cases, the work must be concentrated on giving both parties such conversion factors and methods of calculation that a recomputation of the results can take place in different directions. In order to recalculate the business world's *sortiments*[2] (grades and different piece products of the tree in different dimensions) to the exact cubic volume, various extensive investigations by a special official commission have been carried out in Sweden, the results of which were published in 1923 under the title "Vid Virkensmätning erforderliga relationstal" ("The necessary conversion factors in the measurement of timber"). Representatives of both practical and scientific work participated as members of this committee, the latter group being represented by the writer, among others.

On the other hand, in order partly to develop an estimating system of authoritative soundness suited to standing timber, and partly also to make possible the conversion of scientific results to a more practically useful form, I have been active for 15 years in establishing a system which is now in general use in several Scandinavian countries. This system has also won a certain amount of attention even in America. Since, however, a part of the methods of computation belonging to this system are still unpublished, and are used only in the instruction of my classes and in the national forest survey, which is now in progress in Sweden, I have believed it to be of some interest to present it to this Congress. I shall include, on the one hand, some observations concerning the possibilities of the system, which perhaps have not been sufficiently presented heretofore, and, on the other hand, to make a progress report upon these new methods.

The Investigation of Stem Form

In order to satisfy the demands of practical work for a knowledge not only of the volume of a body of timber, but also giving the diameter of the logs as well as other dimensions, the form of the tree stem must be regarded as of fundamental importance. In fact, I consider the determination of form to be the most important step in the preliminary work. With a knowledge of the form, moreover, the total volume of wood of a certain minimum size, or the amount and dimensions of saw-timber, or of sawn lumber, fuel wood, etc., can easily be determined for trees of all the types that may occur.

As may be known, I have used for the determination of form a method consisting of the measurement of the diameter both at breast height and at each one-tenth of the trunk above breast height, all the other diameters being expressed in percentages of the diameter at breast height. According as the diameter at $^5/_{10}$ or at the center of the bole above breast height formed a greater or less percentage of the diameter breast high, a number of different form classes were made, designated as 0.50, 0.55, 0.60, etc. These "relation factors" have

[2] The Swedish word *sortiment*, will be used in referring to grades and the yield of trees in different kinds of products.

also been called "form quotients" of the stem. As a result of this percentage measurement, together with the separation of trees into form classes, certain relations developed as follows: (1) That trees belonging to the same form class manifested, practically speaking, the same taper, even between all the other points of measurement; this independently of the absolute size, length, etc., of the stem. Large and small trees showed themselves to be constructed according to the same laws. (2) That a number of different species of coniferous trees were so similar in the stem construction that the same series of tapers could be used for a number of species without disadvantage. (3) That the mean taper thus found for different form classes was especially well satisfied by the taper equation developed by the Swedish engineer Höjer, which therefore was used by me for smoothing the results. (4) Lastly, that for a number of species the mean form class may be satisfactorily determined with the help of the so-called form point method worked out by the writer, a method which consists in ascertaining the position of the center of the wind's pressure on the tree crown.

A factor which may disturb the above results to a considerable degree is a more or less extreme localization of branches in the upper portions of the crown, whereby the taper may vary quite considerably both from the average type and from Höjer's taper curve. For the coniferous forest this has shown itself to be of less practical than theoretical importance, but this variable branching habit in a broad-leaved forest in a somewhat open situation may wholly or partially vitiate the advantages of the system, a circumstance, however, with which the writer has occupied himself but little up to the present.

Far more serious, however, is the disturbing influence which is caused by the extension of the basal or root swelling so that this reaches above breast height, the latter being used as a base for the investigation. Where this root swelling is commonly met with only in a few trees, as is most often the case in Sweden, I have recommended for practical use the application of the normal taper series, attempting to eliminate the variation by making the diameter measurement on such trees somewhat higher than ordinary breast height. If, however, as is the case in several places in America, much larger dimensions are found to a great extent, special attention must be paid to this butt swelling which, in general, becomes more pronounced with increasing diameter at breast height. The method which it seems best to follow here is to cast aside completely any thought of using a mathematical equation for taper, and in place use the average figures resulting from the compilation of the data from a large number of sample trees—according to a method used by Maass in his time at the Swedish Forest Experiment Station. That the low-form classes in this way are composed partly of trees with really large taper, and yet partly of trees with good form but large root swelling, has certainly little to recommend itself theoretically, but should be fully satisfactory from the standpoint of the practical use of the results over a number of estimates. I wish therefore to emphasize that the use of my taper series founded on Höjer's equation for all species can in no wise be considered permissible without carrying out special taper studies, at least for certain species and growing conditions. With these, the form class method, in my opinion,

offers greater advantages than any other method now known. I make mention of this especially because recently in Norway a movement has been started to go back to the well-known German system of working with but one mean volume and mean form for all trees of the same height and diameter. On the basis of my own unpublished material with different forest types in Sweden, this in no way satisfies the demands of a good timber estimate, because the form class both at certain ages and also in certain forest types differs essentially from the mean type as determined for a certain height and size.

Finally, touching the proposed form point method, the experience of the past years shows that it is in general well-designed to show the mean form class applying to a stand or a forest type, while, on the contrary, the form of the individual tree, especially those of rarer types, can hardly be said to be brought out in this way. Certain investigations, in part published, and in part still in progress by Tirén,[3] point, however, to the foundation of this method of estimating, that is, the factor of tenacity of the wood, as correct, although the problem may be more complicated than Metzger and the writer first supposed.

Possibly also the continued investigations at the Swedish Forest Experiment Station by Henrik Petterson may give occasion for a revision of the relation between stem form and the form point height.[4]

A more direct way of finding the form class of standing trees, by actually measuring the upper diameter also, now presents itself for at least scientific sample plot investigations. For this purpose a new dendrometer, devised by a Swedish forester, Forstmästare I. Liljenström, seems to give promise of satisfactory results not only for height measurement but also for diameter measurements at every point on the stem where the diameter is clearly discernible. Although I myself have not had opportunity to test the instrument to any extent in practice, I have nevertheless brought the instrument for a demonstration before the Congress because it seems to mark a decided advance in this field.

The Application of Taper Studies

The first result of an investigation of tree form will in general appear to be the finding of the cubic volume and the form factor for different form and height classes. For my own part I consider that the form height, developed by the study, can be most conveniently used; I found that it forms an arithmetical series within each form class, increasing with height. After plotting graphically, the form heights adapt themselves especially well for interpolating and taking out values for any height or form quotient whatsoever. Likewise, the smoothing of the form heights is vastly more convenient than the same operation with form factors, all the more so since experience has shown that very considerable errors may occur in the latter procedure. I have used the smoothed form heights for the construction of general volume tables for trees of different diameters,

[3] Tirén, L. Några undersökningar över stamformen. [Investigations in Tree Form.] *Skogsvårdsför. Tidskr.* **24** (1–2): 23–88. *27 fig.* 1926.

[4] Petterson, Henrik. Sambandet Mellan kronan och stamformen. [The relation between the crown and stem-form.] *Skogsvårdsför. Tidskr.* **23** (1–2): 37–79. *6 fig.* 1925.

heights, and form classes, and these tables have come into use, among other places, in the national forest surveys of Sweden and Norway where the cubic volume of every sample tree is estimated, and from it the volume of the average tree in every diameter class is taken from the arithmetic mean of all the individual tests thus made. For many scientific or practical problems it is, however, simpler to arrive at the average height and form quotient of a diameter class; whereupon the volume of this class can be computed from the basal area and mean form. I consider this to be especially recommended if the volume inside of the bark is desired, since the diameter inside of the bark seldom shows a uniform value suitable for use in volume tables constructed only for certain even classes.

However, volume is seldom sufficient for estimates for industrial and commercial purposes; here the results of taper studies find just their right usefulness in facilitating the calculation of *sortiments*, etc. The importance of such calculations should be apparent from the fact that before the taper tables giving the per cent of the diameter at breast height for every meter of the tree's height had been prepared by me, a very large number of other methods for expressing this relation had arisen, varying according to the differing needs of the special work. By using some of these tables, the diameter and length of saw logs, for instance, can be separated out according to the demands of good utilization of the wood in different tracts, and then even the number of board feet may be found by whatever conventional log rules are employed, this for every log as well as for the tree as a whole. By arriving at the mean height and mean form quotient for each diameter class in forests of a certain type, or on a certain tract, even local taper curves can be plotted according to the system used by Ronge, showing the form and yield in products for the average tree of any breast height dimensions, from which a local table for the tract in question can be obtained.

A totally different kind of factor is demanded in investigations for the paper pulp industry, which above all wishes to know the supply of wood over a certain minimum dimension, as a whole, and without being interested in the length of the timber or other dimensions. The simplest form for such a calculation I have found to be as follows: First compute the total volume, then reduce it by a percentage figure for that part of the volume not usable in the different diameter classes. For the calculation of that part of the wood volume which lies beneath a certain arbitrary size, I published a long time ago so-called "*sortiment* tables" which, however, have been transformed graphically this year for the use of the national forest survey to show the total volume of wood over and under a certain whole-inch class in the top, and where at the same time the annual increment of the same sort of wood can be computed by the same table in the manner to be described below.

The advantages of this system, which at first hand rest on the actual volume of the trunk, afterward reduced to a commercial method of measurement, are apparent in the strong connection which has been found between the wood produced at any period and that which is merchantable, as well in the standardizing of the empirical taper results, so that new calculations may be made at

any time as conditions of utilization change, without necessitating new measurements.

The Calculation of Current Growth

To the extent that production of new timber and new values gains in importance compared with the exploiting of natural supplies, it becomes ever of greater importance that forestry be furnished with methods designed to show the course of increment both as an effect produced by a certain silvicultural treatment, and also on the whole for greater or lesser tracts of forest. Since the subject of growth in our Swedish forests, just on account of this background, is given more attention than perhaps anywhere else, it has been my endeavor to develop methods for the investigation of growth which would take into account both the volume of wood and the yield of different *sortiments* and value. Accordingly, I shall place before you some evidence, especially as a result of the work of the past year.

As an advantageous procedure in the operation of calculating I have chosen the method of seeking to represent the variable quantity as a product of 2 factors whose sizes as well as their annual increases are determined each for itself. The annual increase of a product can be expressed as the differential of the product according to generally known laws of calculus. If thus one factor, a, grows by the amount z_a to A, while the other factor, b, grows by the amount z_b to B, the product ab grows by the amount z_{ab} to AB, and by calculus the increase of the product will be:

$$z_{ab}=Az_b+Bz_a-z_az_b \ (1), \text{ or } =Az_b+bz_a \quad (2), \text{ or}$$
$$z_{ab}=az_b+bz_a+z_az_b \ (3), \text{ or } =az_b+bz_a \quad (4)$$

This derivation shows that if 1 factor refers to the beginning of the growth period, and the other its end, we have thus a convenient formula with but 2 terms.

Not infrequently it is more convenient first to derive the growth in per cent, p, which then is applied to the known growing capital, k or K, as is desired.

$$\text{Growth } z=\frac{p.k}{100} \text{ or Growth } z=\frac{p.K}{100}$$

In obtaining p, different formulae must be derived depending on whether they shall be used with k or with K and since I have worked most thoroughly with that capital existing in the very year of measurement, that is, that capital which comes into being after the laying down of the increment, I have been compelled to derive percentages according to the type:

$$p=\frac{100.z}{K},$$

for which type I have used the name "discount per cent" in distinction to "rebate per cent" which refers to the initial capital, k, thus:

$$p=\frac{100.z}{k}$$

If the percentage growth for the product AB is sought, it is obtained in the same well known manner according to formula (1), thus:

$$p_{ab}=p_a+p_b-\left(\frac{p_a \cdot p_b}{100}\right)$$

where the last factor can often be discarded as being of insignificant size from the practical standpoint.

For calculation of volume growth I have chosen the method of deriving the volume growth in per cent as the sum of the growth per cents of the basal area and the form height, each of which is determined separately. This form of holding separate the growth of both factors has been selected among other reasons because investigations showed that no reliable connection exists between growth in height and growth in diameter, or basal area; therefore all older methods, as for example those of Pressler, Schneider, and several others are of little use for my purpose, the more so since they are not applicable to the final capital, K. For reasons which space does not permit me to discuss here, I have chosen a discount per cent formula of the following type for the growth in basal area p_g:

$$p_g=\frac{100}{n}\left(1-\frac{d^2}{D^2}\right)$$

where D is the present diameter and d the diameter n years ago. After the measurement of D and the width of the annual rings both d^2 and D^2 are obtained from an auxiliary table, or also p_g is found directly from another auxiliary table, which is useful as soon as n refers to a constant period of time, for instance, 10 years. If a number of trees belong to the same diameter, class D, the average width of annual rings, and the average diameter n years ago may then be used advantageously in the formula. For securing the average growth per cent for a number of basal areas of different sizes, on the other hand, the sum of all D^2's and d^2's must be used in the formula. The formula thus changed has its great use partly in the calculation of the growth per cent for the whole stand and partly in determining the volume growth per cent of felled trees when diameter measurements and widths of annual rings are taken by sectioning at even intervals (stem analysis). The same procedure can be used for determining the per cent of bark of sectioned trunks, in which case D and d refer to the diameters outside and inside the bark respectively.

Concerning the growth in form height, its calculation presupposes a possibility of measuring the length of the average leader of the last year. As pointed out before, within the same form class the form height rises in arithmetical series with increasing height. Since it has been shown that the form class, at least for all trees not too young, changes but little (during the period), I have made use partly of the known form height and partly calculated the growth in form height as a function of the growth in height, by which it has been possible to calculate out the per cent growth in form height, as soon as the height of the tree or diameter class, the length of its leader, and the form class are known. The results

are to be found in the auxiliary tables, which, like the tables of basal area growth per cent, are published in the volume tables issued by me.

Since the rôle played by the form class is negligible, growth per cent in form height can be obtained as an approximate value if the length of the leader is considered as a percentage of the length of the entire tree, reduced by 8 feet.

$$\text{Growth per cent in form height} = \frac{100 \times \text{length of leader}}{\text{Length of stem} - 8 \text{ ft.}}$$

For broad-leaved trees and conifers whose leaders are not distinguishable, recourse must be had to another plan, for instance, a preliminary study on felled trees of the relation between volume and basal area growth; time does not permit me, however, to go into this form of procedure.

From the sum of the growth percentages of the basal area and form height, the volume growth per cent is obtained, which gives the absolute annual volume growth by application to the total estimated volume. This method has been used not only in the national forest surveys in Sweden and Norway, but also in connection with a large number of our more recent timber estimates for purposes of management, etc., by which wide experience has been gained on increment conditions over larger forest tracts.

This experience points to a condition not sufficiently appreciated, that the growth per cent is independent of site quality and situation and, by and large, only a function of the age of the stand; this latter function is so constant that empirical percentages can be used to great advantage in the calculation of increment, as soon as the growing stock can be inventoried into age classes of, for instance, an interval of 20 years. Not even different species show any very great deviations in this respect, wherefore in even-aged stands a calculation of the growth per cent by age classes on the part of experiment stations, for instance, can become of great and lasting value as at least a rough estimate of the annual growth in wood content in different parts of the country.

Different Growth of Different Sortiments

These methods of calculation, which contain nothing new, at least in principle, suffer obviously from the same disadvantage as a simple volume estimate of the growing stock; namely, they show nothing about the growth of wood of use for different purposes. My efforts have therefore been directed for many years toward methods of computing the annual production of wood of which a certain quality is demanded, by which one commonly means, as the simplest quality requirement, a certain minimum size in the top. The growth of such a sortiment takes place in such a way that on the one hand a *genuine* growth arises through the formation of new wood around that part of the body of wood previously usable, and on the other hand a certain quantity of wood from a lower size class is transferred to the larger class in question, whereby a *false* growth occurs in this latter class. This last growth becomes at times highly significant, for example, when trees which have previously been below the minimum size

measure are suddenly, with their entire effective volume, lifted over the limit to a merchantable dimension.

The means I have used for the solution of this problem consist in a determination first of the total volume, together with its growth according to the manner previously described. Likewise, with the aid of a graphical *sortiment* table one may read off what per cent of the total volume has attained the desired dimension; this is done for the present and, for example, 10 years ago, when the tree had a height and diameter which can be calculated by aid of the width of the annual rings and the length of the leader as used in finding the volume growth. The difference between these 2 per cent figures indicates the increment or more correctly the change in the frequency figures. It may then be determined what is required for a computation of the absolute increase of the sortiment in question according to one of the formulae given earlier in this paper (1 to 4).

For an illustration of the method, an example of the computation may serve to explain better the procedure. We shall suppose a diameter class 8 inches breast high, containing a total volume of 1000 cubic feet with a current annual increment of 3 per cent, or 30 cubic feet. If a certain *sortiment* can be utilized down to 6 inches in the top and the auxiliary table shows 70 per cent, or 700 cubic feet of such wood, while 10 years ago (with the then existing lower size) only 55 per cent was shown, then the "*sortiment* per cent" for 10 years is thus: $0.70-0.55=0.15$, or 0.015 per year.

From this may be obtained the absolute growth of the *sortiments* according to formula (1), thus:

$$=1000(0.015)+(0.70)\ 30-(0.015)\ 30$$
$$=15+21-4.5=31.5 \text{ cubic feet}$$

Through "false" increment more wood has here been carried to the 6-inch class than actually has been newly formed, and these 31.5 cubic feet make up 4.5 per cent of the supply of such wood, while the growth of the total volume is only 3 per cent.

In addition to the increase in volume on a *sortiment* already in the diameter class, as calculated above, there is added as increment the trees and quantities of top wood which annually surpass the lowest diameter breast high within which the *sortiment* in question is found. If in this way merchantable 6-inch wood can only be taken from trees which are exactly 7-inch breast height, and if the growth in trees of this size is, for instance, 0.1 inch in diameter per year, then roughly $\frac{1}{10}$ of the volume in the 6-inch class passes each year into the larger merchantable class, whose volume must yet be reduced in the manner shown before for the top which is not yet merchantable. In the practical carrying out of these calculations certain corrections must be made in case the wood volume is not evenly distributed among the classes which are just growing into the merchantable class, for the details of which time does not allow consideration here.

These investigations, the results of which are now in practical use for the first time in part in our national forest survey and also in private estimation

work, throw an entirely new light on the production of products for technical use, the growth of which in per cent as well as in absolute measure has many times heretofore been underestimated. In order to emphasize this fact, which is very important in many calculations, I wish to present the following results from an investigation of growth per cent both in total volume and in timber suitable for saw-logs from one of our demonstration forests, which, transformed to a forest with normal distribution of age classes, gives the following results for different rotations:

Rotation years	Mean annual increment per cent	
	In total volume	In saw-logs
80	4.3	6.3
100	3.4	4.4
120	2.9	3.4

These new results have given me the impression that perhaps certain calculations of the forestry situation here in America are possibly needlessly pessimistic concerning growth and cutting, just as I myself was led into the same mistake at an earlier stage.

Annual Value Increment

Even if the growth in total volume and growth in *sortiments* for industries, as previously touched upon, are always of interest, the real question in the end is this: What is the value in money of the wood volume which is annually produced under various conditions?

Earlier it has been possible to answer this question only after very laborious computations, and even then chiefly only where complete yield tables were available; while both for sample plots and larger forest tracts the answer could be given only in the form of a rough approximation. However, during the work of the past year some new points have developed concerning this question which should become of some importance and therefore will be briefly touched upon. I neglect here entirely the increase which can take place by reason of changed market prices and shall consider only the combined quantity and quality increment.

In certain cases calculation of value growth becomes especially simple in direct consequence of the above described investigation of the distribution of growth within different *sortiments*. If the calculation is made of the annual increment, for instance, of sawlogs, or of pulpwood and firewood, the quantities of each produced may quite simply be valued according to the prevailing prices for different products. If, on the other hand, as most often happens in Sweden, larger and smaller wood of the same species is valued at different prices, there is obviously no difficulty in specializing the study to take care of each size class in such a way as is now being done in our Swedish national forest survey where the annual increment for each portion of the country is worked out separately in 1-inch classes according to top diameter, so that the annual increment of 4, 5, 6-inch wood, etc., is given in the final result. In case the price per unit of volume increases with increasing size, that is, if the so-called quality increment is taking place and can be determined for its size, the amount of the total value growth

can also be reduced in other ways; here it is often best to calculate first the increase in volume and thereafter the price or value per unit of volume produced. The derivation of this latter value has puzzled me perhaps more than any other problem with which I have been occupied during the last decade. Without taking the time here, with the possibly troublesome derivation of my calculations, I shall merely give the final formula which is used, showing the value of a unit volume of newly produced wood. If then the diameter of the growing "mother tree" be called D and its stumpage price per unit volume of body-wood Q (the quality factor) and this figure for an increase of one inch in diameter at breast height rises by the amount z to $Q+z$, then one obtains

$$\text{The value of a unit of newly formed growth} = \frac{Q+ZD}{5}$$

This formula which is an approximation, may be illustrated best by an example. If a cubic foot of body-wood from a 12-inch tree is worth 10 cents, while that from a tree 1 inch larger is worth 12 cents per cubic foot, or 2 cents more, then we have:

$$10+\frac{2\times 12}{5}=14.8 \text{ cents.}$$

The formula shows upon analysis that a high value of growth can be obtained if the increase in price with rising diameter occurs rapidly, that is, if the factor z is large; but the original tree ("mother tree") ought also to be of good quality, or have a satisfactory value per unit volume. Good values cannot be created upon damaged and defective "mother trees."

By combining this formula with some other giving the growth in volume, one can easily obtain simple expressions showing the growth in money alone in individual trees, it being necessary to measure only the diameter breast high and to take a boring showing the width of the annual rings.

By means of auxiliary tables of this last kind, one can often stimulate the interest for good handling of the forest far beyond the reach of the forester, and on the whole I have a feeling that in the methods described above I have succeeded in establishing a connection between science and practice which can lead only to advantage, not only for practical commercial life, but also for scientific work and for consideration by practical men.

THE ROLE OF FORESTS IN THE CIRCULATION OF WATER ON THE EARTH'S SURFACE[1]

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From the earliest times there existed among laymen, and even scientists, a belief that forests exercised an influence upon the climate of entire countries. With the introduction of accurate methods of meteorological observations, this popular conception has seemingly been greatly discredited. All that most of the meteorologists were willing to admit was that forests have a local influence upon climate, extending only over the territory actually occupied by them. Within recent years, just when this view seemed to be completely disposed of, many new facts came up independently in different countries, which point strongly to the possibility of the forest exerting a potent influence upon the humidity of regions lying far away from it. Let us consider in the light of these new facts the conditions prevailing in the eastern part of the United States, and see whether there is a relation between the forests of the coastal plain and the southern Appalachians, on the one hand, and the humidity of the central states and prairie region, on the other.

There are 3 fundamental facts upon which, in my judgment, this relation may be based:

(1) In the eastern half of the United States there is a marked periodicity in the wind direction. In winter the prevailing winds are from the north and northwest; in summer the prevailing winds are from the south. When the prevailing winds come from the south the entire eastern half of the United States is wet. When the prevailing winds are from the northwest and west the precipitation decreases. Therefore, the precipitation of the eastern half of the United States depends largely upon the prevailing southerly winds which come from the Gulf and penetrate far into the interior of the continent.

(2) The evaporation from the ocean plays a comparatively unimportant part in the precipitation over the land; $\frac{7}{9}$ of the precipitation over land is supplied by evaporation over the land itself and only $\frac{2}{9}$ is furnished by the evaporation from the ocean. Therefore, the greater the evaporation from the land which is in the path of the prevailing southerly winds, the more moisture must be carried by them into the interior of the continent.

(3) The forest evaporates more water than any vegetative cover and much more than free water surfaces. Therefore, forests enrich with moisture the winds

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 19, 1926.

that pass over them and contribute to the humidity of the regions into which the prevailing air currents pass.

Wind Periodicity and Precipitation in the Eastern Half of the United States

Next to Asia, North America is the largest continent in the world. One of the most striking physiographical features of North America is that the mountains run along the meridians and not along the parallels. The entire northern part of the American continent has no high mountains except in the western part. As the result of this the central part of the continent does not offer any obstruction to winds from the thirtieth to seventieth degree of northern latitude, that is, from the Gulf of Mexico to the Arctic Sea. Even the Asiatic continent does not have such a large continuous area free of mountains extending along the meridian. There the greatest extension is from the thirty-eighth to the seventy-third degree of northern latitude, that is, from the southern border of the plain of Touran to the northern shores of western Siberia. To the south of the thirtieth degree extend the waters of the Gulf of Mexico. The mountains on the southern shore of the gulf begin only at 19 degrees of north latitude. The North American continent, therefore, together with the interior lakes forms an expanse for the movement of the air between the tropical and arctic regions, such as is found outside of it only on large oceans; in the northern hemisphere, on the Atlantic Ocean.

Another climatic peculiarity of the eastern United States which has a bearing upon the question under discussion is the rapid decrease in temperature from south to north. Take, for instance, Labrador; it is entirely an Arctic region where agriculture is impossible. Yet it lies in latitudes at which, in Europe and Asia, agriculture is still flourishing and large populous cities are found (in the fifty-third to the sixtieth degree north latitude are found Christiania and Leningrad). Florida, on the other hand, between the twenty-fifth and thirtieth degree of north latitude, is almost a tropical country. Between Florida and Labrador the drop of temperature for each degree of latitude (60 miles) is for January 2.9°F.; for July 1.08°F.; and for the entire year 1.7°F. Comparing the same latitudes in Europe the drop for each degree of latitude is less than half of that for the North American continent. Between the Canary Islands and northern Scotland, the decrease in the mean annual temperature for one degree of latitude is only 0.8 of a degree.

During winter and partly in the fall and in the early spring, the winds in the eastern part of the North American continent east of the one hundredth meridian come from the west and northwest. These prevailing winds bring cold and comparatively dry air from the interior of the continent. In the spring and early summer these winds are hot and dry. In summer the prevailing winds are from the southeast in Texas, and farther north and east they come from the south and southwest. Henry, in his "Climatology of the United States," says that in midwinter northwesterly winds prevail uniformly over the Missouri Valley and the upper and middle portions of the Mississippi Valley. As the

spring advances the region of southeast to south winds spreads northward and eastward from the Texas coast, so that by April it embraces the states of Texas, Oklahoma, Arkansas, Mississippi, Louisiana, Alabama, western Tennessee, Missouri, Kansas, southeastern Nebraska, and Iowa. By June the northwest winds of midwinter have been supplanted by southerly winds over practically the whole of the country east of the Rocky Mountains. In autumn the northwest winds become more frequent, and as autumn shades into winter they gain the ascendency in the Missouri and Mississippi Valleys and in the plains states.

The periodicity is clear when one examines Weather Bureau maps based on 20 years' observation, on which, by arrows, is indicated the direction of the prevailing winds, and by lines the mean precipitation for the months of July and January. The map for the month of July is typical for the summer period and the one for the month of January is typical for the winter period. These maps show, very clearly, that the eastern half of the United States is under the influence of 2 prevailing winds; one, which originates in the Gulf of Mexico and in the Atlantic Ocean, is mild and humid; the other, which comes from the interior of the continent and from the Rocky Mountain region, is dry and continental in character, that is, dry and cold in winter and dry and hot in the spring and summer.

Another important fact which the records of precipitation and wind direction establish is that there is a most intimate relation between the prevailing southerly winds and precipitation in the eastern half of the United States. It is during the summer period, when the entire eastern half of the United States is under the influence of the southerly winds, that most of the precipitation falls over it. On the plains east of the Rocky Mountains the summer rainfall forms from $\frac{3}{4}$ to $\frac{4}{5}$ of that of the entire year. In July when the prevailing southerly, southwesterly, and southeasterly winds extend far into the interior of the continent as far north as North Dakota, and as far west as the foothills of the Rocky Mountains, and even into eastern New Mexico, and as far east as New England, the precipitation over the entire eastern half of the United States is very heavy. In winter the picture of both wind direction and precipitation is radically changed. The northerly and northwesterly winds have not the same pronounced persistence as the summer winds. Yet through the entire south—Texas, Louisiana, and Mississippi—as well as the Atlantic states, the lake states, and the central states, the prevailing winds are northerly and northwesterly winds. At the same time there is a perceptible decrease in precipitation through the entire eastern half of the United States, and where in July there fell as much as 3 inches of rain, in January there is less than 1 inch, and where in July there fell as much as 5 inches there is in January less than 2 inches.

This increase and decrease in precipitation over the eastern half of the United States, with change in the direction of the wind, points to the fact that the eastern half of the United States depends for its moisture upon the prevailing southerly winds, which originate in the Gulf of Mexico and the Atlantic Ocean.

The central interior region of the United States is thus the battleground of 2 titanic forces, of which one is harmful and the other is beneficial. The

beneficial force takes its origin in the Gulf of Mexico and the adjoining ocean, the harmful in the interior of the continent and the Rocky Mountain region, and whether it comes as the warm chinook winds which blow out of the northern Rocky Mountains, or as the dry westerly winds of the Upper Mississippi Valley and the western lake region, occurring especially in the spring and early summer, it always carries in its wake serious injury to orchards and fields.

The central states and the prairie region are geographically at the point where the battle between the two forces is fiercest and the victory is now on the one side and now on the other, being dependent upon the cold and humid, and the warm and dry, climatic cycles as well as upon the seasons of the year.

When the humid southerly winds extend their influence far into the interior of the continent, and overpower the dry continental winds, the central states and prairie region, the granary of the United States, produce large crops. When the dry winds overpower the humid southerly winds there are droughts and crop failures.

The southerly winds on their way from the Gulf of Mexico do not meet any mechanical obstructions. Since the Appalachian Mountains, running in a northeasterly and southwesterly direction, do not hamper their passage, they are capable of penetrating far into the interior of the country and, therefore, determine the amount of precipitation, even in such states as Minnesota, Nebraska, North and South Dakota. The moisture-laden winds from the gulf, as soon as they reach the land and encounter irregularities, are cooled and begin to lose part of their moisture in the form of precipitation.

As long as the air currents are saturated with moisture the slightest cooling or irregularity of the land that causes them to rise will cause precipitation. But as they move inland and become drier the remaining moisture is given off with difficulty and precipitation decreases. The sooner the humid air currents in their passage over land are drained of their moisture, the shorter is the distance from the ocean over which abundant precipitation falls; the longer the moisture is retained in the air currents, the farther into the interior will it be carried and the larger will be the area over which precipitation will be distributed.

If precipitation over land depended only on the amount of water directly brought by the prevailing humid winds from the ocean, the land would be pretty arid and rainfall would be confined to only a narrow belt close to the ocean. Fortunately, not all the water that is precipitated is lost from the air currents; a part runs off into the rivers or percolates into the ground, but a large part of it is again evaporated into the atmosphere. The moisture-laden currents, therefore, upon entering land at first lose the moisture which they obtained directly from the ocean, but in their farther movement into the interior they absorb the evaporation from the land. Hence the farther from the ocean the greater is the part of the moisture of the air contributed by evaporation from the land. At a certain distance from the ocean practically all of the moisture of the air must consist of the moisture obtained by evaporation from the land. At least it must form a larger part than the water which was obtained directly by evaporation from the ocean.

The vapor brought by the prevailing winds from the ocean is many times turned over or reinvested before it is returned again to the ocean through the rivers.

If we could reduce the surface run-off, and at its expense increase the evaporation from the land, we should thereby increase the moisture of the passing air currents, and in this way contribute to the precipitation of that region into which the prevailing winds blow. This conclusion is almost axiomatic.

"Continental" and "Ocean" Vapor

For a long time it has been accepted without any question that all the vapor that is condensed in the form of rain or snow over the land surface is furnished by the evaporation of water from the oceans.

The part which vapor from the ocean plays in the precipitation over land has been altogether exaggerated. Professor Brueckner[2] has computed the amount of water evaporated from the ocean surface, land surface, and the amount of water which is returned to the oceans and the land in the form of precipitation. The balance sheet of the circulation of water on the earth's surface is made up as follows:

TABLE 1. CIRCULATION OF WATER ON THE EARTH'S SURFACE

Balance Sheet

	Cu. miles, Vapor	Depth, inches	Per cent
A. Entire earth's surface (196,911,000 sq. miles)			
Evaporation from water surfaces	92,121	29.5	80
Evaporation from land surfaces	+23,270	7.5	20
Precipitation on entire earth's surface	115,391	37.0	100
B. Oceans (141,312,600 square miles)			
Evaporation from oceans	92,121	41.3	100
Amount of ocean vapor carried to the land (net[3])	5,997	2.8	7
	86,124	38.5	93
C. Peripheral land area (44,015,400 square miles)			
Ocean vapor (net)	5,997	8.7	29
Continental vapor from the peripheral land surface	20,871	29.9	100
Precipitation over the peripheral land area	26,868	38.6	129
D. Closed interior basins with no drainage to the ocean (11,583,000 miles)			
Evaporation from closed basins	2,399	13.0	100
Precipitation over closed basins	2,399	13.0	100

The continental vapor which is fed from the periphery of the land surface is thus about 21,000 cubic miles. It plays, therefore, an important part in supplying the moisture to the air, even a more important part than the vapor directly fed from the ocean. The peripheral regions of the continents, that is, the regions

[2] Brueckner, Eduard. Die Bilanz des Kreislaufs des Wassers auf der Erde. Geographische Zeitsch. **11** (8): 10 pp. 1905.

[3] This refers to the difference between the amount of vapor that escapes from land to the ocean and from the ocean to land.

tributary to oceans are capable of supplying $\frac{7}{9}$ of their precipitation by evaporation from their own areas. The moisture which is carried by the winds into the interior of vast continents, thousands of miles from the ocean, is almost exclusively due to continental vapors and not to evaporation from the ocean.

In the interior enclosed basins, the precipitation and evaporation, as a rule, are equal to each other.

Brueckner's figures for the entire earth's surface are corroborated also by studies of specific drainage areas. The most interesting studies in this connection are those by Francis E. Nipher[4] and George A. Lindsay[5] on the rainfall of the state of Missouri and the discharge of the Mississippi River at St. Louis and at Carrollton, Louisiana. Nipher found that the average discharge of the Mississippi River at St. Louis during the ten years ending December 31, 1887, was 190,800 cubic feet per second. The amount of water falling per second upon the whole state during the same interval was 195,800 cubic feet, equal within 2 per cent to the discharge of the Mississippi River at St. Louis. If, however, a comparison is made between the total rainfall on the basin draining past St. Louis and the river discharge at this point, it appears that the drainage area of the Mississippi and Missouri Rivers above St. Louis is 733,120 square miles, or over 10 times the area of Missouri. These figures show what a small portion of the total rainfall over the drainage basin of the Mississippi River is led into the rivers and conducted back to the sea. It is evident that by far the larger portion of the precipitation that falls over the drainage basin is evaporated back from the land into the atmosphere, and is not returned to the sea through the medium of drainage. These figures show further that the source of precipitation of the Mississippi drainage is from evaporation over the land and not derived from evaporation over the sea. Lindsay computed the discharge of the Mississippi River at Carrollton, Louisiana, and found that the average for 14 years was 117 cubic miles per year, or 545,800 cubic feet per second, which is less than 3 times the precipitation over the state of Missouri.

The central portion of the United States is distinctly a continental region, particularly the prairie region, which suffers from lack of precipitation. On the other hand, large areas in the South and Southeast suffer from too much moisture because of large swamps. This excess is caused not only by excessive precipitation but also by deficient evaporation. Not only the southern and southeastern areas suffer from too much water but also many portions in the North and Northeast, where the evaporation is also very slight. We have, therefore, 2 extremes on the periphery of the United States: (1) in the states adjoining the Atlantic Ocean and the Gulf of Mexico there is an excess of moisture on the ground, both on account of excessive precipitation and slight evaporation; (2) in the vast interior of the central United States, on the other hand, there is a deficiency

[4] Nipher, Francis E. Report on Missouri rainfall, with averages for 10 years ending Dec. 1887. Transactions Acad. Sci., St. Louis, **5**: p. 383.

[5] Lindsay, Geo. A. The annual rainfall and temperature of the United States, Transactions Acad. Sci., St. Louis, June, 1912.

of moisture, both on account of the scant precipitation and of the intense evaporation. Is there not some connection between these two extremes? Is it not possible that changes which take place in one part of this vast region may exert some influence on the condition of the other? We have seen that in the central states in summer the prevailing westerly and northwesterly winds give way to the southerly and southeasterly winds. In other words, in the summer the central states are under the influence of moist winds, just at the time when the evaporation is the greatest and the forest vegetation is especially active. It seems, therefore, that the amount of moisture evaporated within the more moist region of the United States can influence the conditions of humidity, not only in the states close to the ocean, but also in the region into which the prevailing moist winds flow. The more moisture there is evaporated from the ground in the southern and southeastern portions of the United States, the moister must be the air in the central states and the more precipitation must fall there.

Forest the Greatest Evaporator of Water

What are the sources from which the evaporation on land is the greatest? The evaporation from a moist, bare soil, is, on the whole, greater than from a water surface, especially during the warm season of the year when the surface of the soil is heated. A soil covered with vegetation loses moisture, both through direct evaportation and through plant absorption, much faster than bare, moist soil, and compared with a free water surface there is a still greater loss.

The more highly developed the vegetative cover, the faster is moisture extracted from the soil and given off into the air. The forest in this respect is the greatest desiccator of water in the ground.

Forest trees require from 500 to 1000 units of water to produce one unit of dry substance. A mature forest produces at least 100 cubic feet of dry substance including leaves, roots, etc., per acre per year. At average specific gravity, 25 pounds per cubic foot for coniferous wood and 40 pounds for hardwoods, this would mean a production of from 2,500 to 4,000 pounds of wood substance per acre per year. To produce this amount, from 2,500,000 to 4,000,000 pounds of water must pass through the trees and be given off into the air. If this amount of water were distributed over 1 acre, it would cover it to a height of 12 inches. These figures, while only approximate, give an idea of the enormous quantities of water given off by forests into the air, which has justly given them the name of the "oceans of the continent."

The most valuable and complete work on the subject is by Otozky, a Russian geologist and soil physicist. This appeared as a publication of the forest experiment stations. Otozky worked up an enormous number of observations, both his personal observations and those furnished him by others, and he found no single contradictory fact. His conclusion is that the forest, on account of its excessive transpiration, consumes more moisture, all other conditions being equal, than a similar area bare of vegetation or covered with some herbaceous vegetation. The amount of water consumed by forests is nearly equal to the total

annual precipitation; in cold and humid regions it is somewhat below this amount and in warmer and dry regions it is above it.

This enormous amount of moisture given off into the air by the forest, which may be compared to clouds of exhaust steam thrown into the atmosphere, must play an important part in the economy of nature.

If the present area occupied by forests in the Atlantic plain and the Appalachian region were instead occupied by a large body of water, no meteorologist would hesitate for a moment to admit that the water surface has a perceptible influence upon the humidity of the central states and the prairie region. Should not, therefore, forests which give off into the atmosphere much larger quantities of moisture than a free water surface, have at least a similar influence upon the regions into which the prevailing air currents flow?

If the southern and southeastern winds, in their passage toward the north, northwest, and northeast, in the spring and summer, did not encounter the vast forest areas bordering the shores of the Gulf of Mexico and the Atlantic coast and those of the southern Appalachian, and, therefore, were not enriched with enormous quantities of moisture given off by them, the precipitation in the central states and in the prairie region would undoubtedly be much smaller than it is now.

What would be the effect of complete or even partial destruction of forests in the Atlantic plain and in the southern Appalachian Mountains upon the humidity of the continental portion of the United States? As the mean temperature in the eastern part of the United States drops rapidly from south to north, the moisture-laden air currents upon entering land would be cooled off, and rapidly drained of their moisture within a comparatively short distance from the ocean. The sandy soil which is so characteristic of the southern pine belt of the gulf and south Atlantic states would rapidly absorb the rain which would percolate into the ground, without returning much of it into the atmosphere. The rain falling upon the slopes of the mountains would rapidly run off into streams. While direct evaporation from the ground not sheltered by forest cover may become greater, yet the more rapid run-off and the absence of transpiration by trees would necessarily reduce the total amount of water evaporated into the atmosphere. If the land were all used for agriculture, it would not return such large quantities of rain into the atmosphere as the forests do. The inevitable result would be that less moisture would be carried by the prevailing winds into the interior of the country, and therefore less precipitation would occur there.

The effect of forests upon climate, if viewed as a local influence, must necessarily be insignificant. First we must not forget that whenever we compare a forest with an open field adjoining it, the open field itself is under the influence of the forest and can not give a proper conception of the true effect of the forest.

Such a meteorological authority as Lorenz Liburnau, at the end of his monumental work on "The Results of Forest Meteorological Observations" remarks that his data and conclusions apply only to the influence which the forest exerts while it exists, but do not extend to conditions which may arise upon its com-

plete destruction. It is stated: "If, for instance, according to our observations in the Carpathian foothills, it appears that the influence of the forest upon the neighboring country is only insignificant, this does not indicate that a complete destruction of all the existing forests will produce here also only insignificant climatic changes. Very likely that, if the forest were completely destroyed, the difference would be much greater than the difference that exists now between the climate of the forest and its neighboring areas."

Local observations, no matter how accurately and minutely carried out, can not lead us to the solution of the problem. The method of attack itself is wrong. It is only by approaching the problem from a much broader standpoint, by rising mentally to a height which opens wide perspectives both to the distant shores of the Gulf of Mexico and the Atlantic Ocean and to the most interior portions of the continent; only by following the moist south winds on their way from the gulf through the gateway of the North American continent, the Atlantic plain to the Prairie region, by considering how many times the moisture carried by the wind is dropped in the form of precipitation and raised again as evaporation, by studying the part which the vegetative cover plays in this circulation of water on the land, especially the dense coniferous forests, that we can grasp the problem in its true light.

THE IMPROVEMENT AND DEVELOPMENT OF THE FORESTS OF INDIA BY MEANS OF SCIENTIFIC RESEARCH[1]

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The Secretary of the Program Committee has been kind enough to ask me on behalf of his committee to send a paper for this Congress, the subject to have some general bearing on the topic "The scientific foundation of forestry as exemplified by forest experiment station work."

The title of my paper will indicate the lines on which I propose to treat the subject, which is of peculiar interest to us in the Indian Forest Service. This service is probably the most highly developed and most numerous of all forest services in the British Empire, and has now under its charge approximately 230,000 square miles, more than $\frac{1}{5}$ of the total area of British India.

It has been realized, almost since the beginning of the British occupation of India, that the welfare of the country depended to a considerable extent on the proper conservation of its forests and on a strong forest policy. It is unnecessary to dilate upon the fact, well known to all students of Nature, that the welfare of forests has been, as a rule, a matter of entire indifference to primitive races, which regard forests as a rule much in the same way as they regard water, the sun, and the earth, that is, as inexhaustible sources of the bounties of Nature. Fortunately, there were during the last century men in India who realized what forests might mean to the Empire. A report was made on the forest resources of Tenasserrim 100 years ago and in 1842 one of the magistrates in the Madras Presidency was enlightened enough to start the now famous Nilambur teak plantations.

In 1885 the Viceroy, Lord Dalhousie, laid down a definite forest policy and Dr. Brandis, who later became the first Inspector-General of Forests to the Government of India, was appointed Superintendent of Forests in Pegu (Lower Burma) in 1856. Strong opposition, not only from the various races of India, but also on the part of short-sighted district officials, marked the birth of the Forest Department in most provinces, but sound policy eventually prevailed, and the forest estate is now administered by about 600 trained forest officers, under whom work about 15,000 subordinates, rangers, deputy rangers, and forest guards.

The forest estate in their care is made up of forests of infinite variety lying between 8° and 34° of north latitude, including the fine deodar, pine, and spruce

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca New York, Aug. 20,1926.

forests of the Himalayas, the very valuable forests of teak (*Tectona grandis*) and Sal (*Shorea robusta*), the magnificent evergreen forests of Madras, Burma, and Assam, the small scrub of the dry plains of the Punjab and Rajputana, and many other vegetative types.

At first the work of the department was confined to exploration, demarcation, and settlement combined with such exploitation as could be safely undertaken in order to make use of the mature timber and other products for the benefit of the country, and for the realization of revenue. As the forests were settled and surveyed, correct management became yearly of greater importance and special attention was paid to the compilation of scientific working plans. Later still, forest officers realized that organized research was a necessity if the forests were to be subjected to a progressive régime of improvement and development. It may be considered that the Forest Department of the last century should be blamed for not starting scientific research, but it must be remembered that the officials of those days were struggling to prove that the forests were really an asset to India and to justify their existence by financial results, which should appeal to the bureaucratic mind. It is true that a few valuable scientific investigations were carried out by individuals, but the results were meager because system had not been introduced.

About the year 1900 the first research officer was appointed. Some of the senior officers in the department had been struck by the extent to which valuable forests were damaged by insects and this led to the appointment of a forest zoologist. His work began on a small scale, and he did not always receive the commendation of the higher officials, as it is recorded that one of them was indignant because no literature had been produced after one year's work! However, research was coming into its own, and the first forest experiment station took shape at Dehra Dun in the north of India, in 1906–7. Five branches of research were recognized, and officers were appointed to take charge of these branches, which are still in being in 1926, silviculture, economy (utilization), entomology, botany, and chemistry. As a justification for the expenditure incurred in research it may be noted that the gross revenue of the forests in 1923–24 was about £4,000,000, of which £2,500,000 went into expenditure. It is fair to credit to forest research in India a substantial proportion of the surplus, which increased from £600,000 in 1900–01, to £1,500,000 in 1923–24.

The various branches of research have contributed in different measures to the growth of the value of the forests as a great asset for India, and it is worthy of note that every endeavor has been made to keep the various branches in touch with one another, and to see that the work done by any one branch supplements that done by the others.

Although the headquarters of forest research for India are at Dehra Dun, a great deal of important research is also carried out in the provinces. The most important forest provinces are Madras, Burma, Bombay, Punjab, the Central Provinces and the United Provinces, and 4 of these have silviculturists. Bengal and the province of Behar and Orissa also have silviculturists. Madras and Burma have officers in charge of utilization.

The work done by all these officers is coordinated with that done at Dehra Dun. In a country of immense distances it is obviously not advisable to centralize all research at one point, but the present arrangements are found to work well, and the advice and cooperation of the Central Institute are frequently sought by local officers.

When describing the work done by research officers it should be remembered that one of the most striking characteristics of the forests of India is that they seldom consist of pure stands of one or two species. As a rule a valuable timber grows mixed with many others which are of little or no value at present, and it will be readily understood that the silvicultural and economic management of such forests offers very special problems. There are of course exceptions, such as the coniferous forests of the Himalayas, the forests of *Shorea robusta* of Northern India, and the forests of *Dipterocarpus tuberculatus* of Burma, but they cover comparatively small areas.

The mainspring of silvicultural research has always been to increase the productivity of the forests and to aim at the realization of a sustained yield of valuable timber by introducing correct silvicultural systems. As a natural result of this policy there has appeared of recent years a strong tendency to adopt wherever possible a more uniform method of working, exemplified by the concentration on certain defined areas of exploitation and regeneration. A large part of the time of the silvicultural staff has been occupied in the selection, demarcation, and measurement of sample plots in forest stands of all kinds and ages, and there are now thousands of these sample plots scattered over India and Burma. The records for these plots are maintained in standard forms and in great detail. The figures obtained are as a rule worked up at the Central Institute, and yield tables and other records are prepared as soon as the data available are sufficient. These have proved of the utmost value to working plan officers and to divisional forest officers in the economic management and improvement of their forests. Equally important, and of engrossing interest, have been, especially of recent years, the investigations into the most advantageous methods of regeneration. Natural regeneration as a means of replacing mature woods, has received its due share of attention, but even more work has been put into experimental sowing and planting. Nursery methods have improved enormously, due to the investigations of the silviculturists, and direct sowing and transplanting have been greatly developed in the light of the experience gained all over India during the last ten years. As an example of the problems confronting the silviculturists may be mentioned the use of the seed of the Dipterocarp family, which contains many very important timber trees, nearly all of which bear seed which loses its vitality very rapidly. Good progress is being made with this problem, but it is by no means solved.

Afforestation and reafforestation, also most vital problems in the hot bare lands of India, have been most successfully pushed forward by the silviculturists. It is no exaggeration to say that scientific forest management throughout India today is based on research in silviculture. A magnificent work in 3 volumes, "The Silviculture of Indian Trees" by Troup, who is now professor of forestry

at Oxford, was published in 1921. Troup was the originator of systematized silvicultural research in India and his book contains all the silvicultural information available for Indian trees up to 1920. I may here quote from a small volume "India's Forest Wealth" recently written by E. A. Smythies, I.F.S., and published by the Oxford University Press.

"The heavily overworked and semi-ruined forests of 50 years ago have, with care, protection, and the wonderful recuperative power of many Indian species, been converted into flourishing and vigorous crops, and annual tendings, cleanings, thinnings, climber cutting, etc., still continue the good work. As a concrete example, the yield capacity of the valuable Sal forests of the United Provinces and Bengal have increased 5 to 10-fold in the last 50 years, and there must inevitably be a further considerable increase in the next 50 years."

Turning to forest economy, or utilization of forest products, good progress in experimental work can also be recorded, and to this branch more than to any other we owe the great development that is taking place in the utilization of the forests of India. The greater part of the research in forest economy has been carried out at Dehra Dun, but Burma, Madras, and other provinces have also done a great deal of good work.

Dealing with the more profitable utilization of the timbers of India research has followed the following main lines: Estimation of strength values, wood preservation, seasoning, and wood working.

A large timber testing laboratory has now been in full operation for some years under a Canadian specialist. About a dozen modern machines, mostly from the United States are in operation and more than 100,000 tests have been carried out. The program of tests, conducted by standard methods, is building up a mass of data about all the principal timbers by means of which the uses to which they can be profitably put are being ascertained. Strength values are compared with those of well known foreign timbers, and Indian timbers are reported on for airplanes, bridges, railway-wagons, house-building and many other purposes. The results of these tests have proved of great value to users, and have shown decisively that many Indian timbers have qualities they had not been suspected of possessing.

In considering wood preservation I may quote a paragraph from a paper by John D. Rue in the "American Journal of Forestry" for March, 1926.

"A ready market for ties cut from lodgepole pine, and from second-growth southern pines, and from western hemlock, as well as other species, is greatly limited because of their non-durability, a defect which is, however, overcome by the application of preservatives. Research has determined the chemicals to use and the method of application so that now the non-durable species are in demand and constitute a constructive factor in the forest program."

Allowing for the species being different, this is true, word for word, for India, and is evidence of how the aims of scientific forest utilization coincide in various parts of the world. Railway sleepers are a tremendous problem in India, and the white-ant, the arch-enemy, must be defeated. As a result of 10 years of

research we are now able to recommend to railway companies for sleepers cheap timbers which can be treated and made very serviceable. Other lines which have been investigated are railway-wagon timbers, piles, fenceposts, etc.

By research it has been already proved over and over again that correct scientific methods of artificial seasoning will make available for use many Indian timbers which have been up to date regarded as little better than rubbish. Timber in India has been in the past very often shamefully misused, so that even the best kinds have in many cases deteriorated before use, while the inferior kinds have never had a chance to prove their value. Systematic experimental seasoning of Indian timbers has been and is being carried on, so that data may become available for all timbers that are, or may be, marketable.

At several centers in India wood-working workshops have been established where the qualities of Indian timbers as regards working are being fully tested. Modern machines, tools, and methods are in use, and it is already possible to furnish information to Government and private users so that the timbers may be used in such manner as to bring out their best qualities.

Hand in hand with the knowledge of the qualities of timbers goes the study of its structure. The study of wood technology was begun in India by H. P. Brown, of Syracuse University and he has produced an invaluable manual for students. A larger work is now in preparation by Brown and by R. S. Pearson. It is to the last-named that the greater part of the credit for the great development of research in forest economy is due.

Besides timber the forests of India contain a wealth of other valuable products. The bulk of the world's output of lac is obtained from India, the annual outturn being worth more than £7,000,000, and besides this there are innumerable products which are now the subject of investigation, such as resins, oils, gums, fibers, dye- and tanstuffs, drugs, spices, edible products, fodder plants, canes, and bamboos. In the province of Burma alone there are some 3,000 woody species, and as there are few of these which yield nothing of use to man, the wealth of India in these products can be imagined.

Bamboos deserve special mention. Apart from the fact that they are extremely useful to the inhabitants of almost every part of India in their daily life, it has now been proved that they can produce a first class paper-pulp at a cost which will make the establishment of pulp mills in India a commercial possibility. Fifteen years of research at the Forest Research Institute at Dehra Dun have borne fruit, and there are at this moment several large schemes under consideration for utilizing the innumerable millions of bamboos in the forests of India. A short account may now be given of the three minor branches of forest research in India, entomology, botany, and chemistry.

It is probably the case that there is not a single tree of importance in India which is not attacked by some insect. In the case of the Sal borer, the teak bee-hole borer, and several others, it is easy to prove that the loss caused by these pests runs into many thousands of pounds annually. The scientists of the entomological branch are investigating these problems very thoroughly, and have already been able to demonstrate that the control on a large scale of an

insect which damages forests may be a profitable operation. Systematic entomology, which is intimately related to the economic question, is also being very comprehensively studied.

The work done in the botanical and chemical branches is to a considerable extent complementary to that of the other branches. Systematic botany has made great strides, though want of staff has prevented progress to a certain extent. The care of a very large herbarium and the study of the germination of seeds of forest importance, have provided full employment for the small staff of this branch. In the chemical branch, work has been principally carried on for the assistance of the silvicultural and economy branches, in the study of forest soils, in the analysis of wood preservatives, of forest plants which produce minor products, etc.

Research on the above lines covers an ever growing field and the whole of the results obtained are put at the disposal of the public and of the various services with the least possible delay, in publications of which about two hundred have already been issued, and numerous inquiries are received from every part of India, and other countries also, regarding forest problems.

PROBLEMS IN AMERICAN FOREST EXPERIMENT STATION DEVELOPMENT[1]

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There has been purposely omitted the mere catalogue of the local problems, or even a catalogue of the major lines of work, as foresters generally recognize in silvicultural research that methods of cutting, fire, and other forms of protection, forest influences, mensuration, and the like are prominent, important, and necessary.

There are 4 major problems all arising from the development of silvical research. The development of the stations; the necessity of considering the forest as a biological unit; the need of an adequate personnel; and the correlation of activities to fit the various component parts into an harmonious whole.

To provide itself with an adequate supply of forest products, the nation has a tremendous task ahead, a task far more difficult than generally realized, even by otherwise well-informed foresters. Already there has been burned or cut over by far the greater part of the original timbered area, much of which is now unproductive, and a still greater part of this area is only partially productive. With the present use of wood, amounting to about 26,000,000,000 cubic feet annually, and with a growth of only about 6,000,000,000 cubic feet it will not be long before the nation will have reached that point where there will no longer be a reservoir supply of old-growth timber to draw upon, and, unless there is grown a large part of the 20,000,000,000 cubic feet needed to meet current needs, it will be necessary either to reduce our standards of living or to import from far distant countries and at high cost the timber we require.

The immensity of our forest problem can only be realized when it is remembered that the forest area includes approximately one-fourth of the total land area of the country, that we are cutting heavily into the remaining 137,000,000 acres of virgin timber, and that to the more than 80,000,000 acres of nonproductive land, there is being added annually additional thousands of acres. We also have many different climates, an innumerable variety of soils, and tremendous physiographic and topographic differences. When to these complex physical conditions are added the complications in forest management caused by the fact that we have over 100 commercially important tree species, that these important trees occur in various proportions, with over 200 non-merchantable species, and that we have many varied insect pests, major and minor forest tree diseases,

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 20, 1926.

that we must contend with grazing both of domestic livestock and game animals, and that we have varied and complex forms of wild life, the magnitude of our task looms even more prodigious. We are also confronted by the time element as a major factor, for the forest crop requires several decades to become mature, while the national timber need becomes more acute with each passing year. To raise the timber crop vital for our needs requires that an adequate and thorough knowledge of the principles of timber growing and forest protection shall be obtained, and that soon for each of our many forest types.

To raise a timber crop both sufficient and in time to meet the national need requires an accurate and thorough knowledge of the principles of timber growing and forest protection in each of our forest types. The establishment, maintenance, and protection of the forest crop can not be left to chance with any more assurance that the final yield will be sufficient to pay for the harvest than would be the case were an agricultural crop similarly treated. Through long years of effort, at first by the costly time-consuming and generally unsatisfactory trial-and-error method, and then later by intensive investigation at well-manned and well-equipped experiment stations, scientific agriculture has been developed. Experience has shown that these stations have been the cheapest and most effective means of determining basic crop production methods. A former cabinet member, a Secretary of Agriculture, recently said that "during the 50 years that the Agricultural Experiment Stations had been in existence, agriculture had made a greater progress than it did in the thousands of years before they had been established." Similarly, and only through the establishment of adequate forest experiment stations can there be furnished in the shortest possible time and at the lowest cost that basic information necessary to solve the problem of how to provide a continued supply of forest products.

The original concept of the forest experiment station was an establishment located in the woods and in connection with one of the busy national forests, one such station for each of the national forest districts of the Forest Service. These original stations, very local in character, were manned by from 1 to 3 men, and for the most part were directly concerned only with the immediate problem of local silviculture on the national forests where established. Most of these stations were located also where nurseries were established in order that the stations could work upon the local problems of forest planting which confronted the Forest Service organization at that time.

The available funds for the most part were meager, necessitating rigid economy in the work of the station, poor equipment, inadequate personnel, and very limited travel. In spite of the handicaps, appreciable progress was made in laying the groundwork for silvicultural management of forests, some of the results of the investigations being applied in the present cutting on the national forests.

In the East, there were no forest experiment stations, the investigative work being centered in Washington with a corps of men who worked out from Washington into the nearby states. Most of this early work was done with reference to the silviculture of the more important forest types in the East, but it is indeed

surprising when one looks back, to find that so many types were covered and that so much work has been done. From the forests of Maine to the swamps of Florida, and from the woodlots of Iowa and Nebraska to the pine barrens of New Jersey, the investigative arm of the Forest Service extended. It even went beyond the bounds of the country proper and covered such an out-of-the-way place as Porto Rico.

About 10 years after investigative work became a recognized feature of the Forest Service there came the European war. War activities necessitated the shut-down of stations, the reduction of personnel, and a general reduction in silvicultural investigations. Following the war, the threads which had been dropped were picked up. At this time also there developed a national realization of the need for research in all lines of human enterprise. Recognizing this, during the winter of 1920 and 1921, the Department of Agriculture announced its general program for the establishment of regional forest experiment stations throughout the country. At that time it was recognized that the federal government had a duty not alone to the western regions where the national foresters were so largely centered, but also to timberland owners in the eastern States where the larger percentage of high-grade potential forest lands lay. Provision was therefore made in the plan for 5 stations to serve the East, and 6 in the West, the West, as before, being divided into 6 regions corresponding to the district organization of the Forest Service and with a reduction in the work carried on in Washington. No longer were the western forest experiment stations to serve only local national forest needs, but they were also to help the private timberland owner and operator solve some of their problems of forest production and protection. They were to work upon regional rather than local problems. With this plan of station establishment was also outlined a general program of work, including investigations in forest protection, forest influences, forest management, planting and nursery practice, and growth and yield.

Considerable interest was evidenced in this plan and shortly after its announcement Congress provided money for starting 2 stations: 1 in the Appalachian region and 1 in the southern States. Since that time additional stations have been established; 2 more in the East in 1923, in the lake states and in the Northeast, and authorization for 2 more stations granted this year, 1 for the Middle Atlantic states and 1 for the Ohio-Mississippi Valley section. In addition, 2 western regional stations have been established, 1 in the Pacific Northwest in 1924, and 1 in California in 1926.

In 1925 a new forest experiment station plan was formulated in the department and a station for the Ohio-Mississippi Valley region included. In the new plan provision was made not alone for establishing regional forest experiment stations within the continental United States, but for extending the plan to 2 national extremes, Alaska and the American tropics, the station for the latter to include work in the tropical forests in the West Indies and in the Canal Zone. When this revised plan was outlined, attention was also directed to the fact that none of the forest experiment stations is as yet adequately manned or equipped to handle the immense timber growing problems of the regions. Congress,

appreciating this fact, has now granted to the Southern and to the Appalachian stations increases which in effect have doubled the number of the personnel.

The first phase of the program of the Department of Agriculture is well on its way, that of establishing the stations; and, from past experience, it would appear that in the space of a few years we may look forward to seeing the local stations yet in existence (in the Rocky Mountains and in the Southwest) placed on a regional basis, and stations established in the 2 eastern regions not as yet furnished with them.

As indicated, the present force of the forest experiment stations has not yet been put on an adequate basis. It is unreasonable to expect that a staff of from 3 to 7 men could successfully solve the problem of timber growing and protection in a region of up to 140,000,000 acres of forest land. It will therefore be necessary to enlarge the present stations to such a size that they will be able to carry on their work properly. The thought has been that to accomplish this would require the services of some 12 to 15 or more men per region, doubling in size even the largest of the present stations.

So much for the future in the development of stations. The establishment of the stations, their expansion to a size adequate to handle the regional problems, bring to the fore the other problems. What is to be done and who shall do it? It is first of all necessary to get a clear concept of the forest, and it is not certain that foresters generally have a clear picture of what the forest really is. To most woodsmen, the forest is merely a stand of trees from which certain individuals are to be cut at stated periods, a stand which must be protected from fire, which must be developed for the tourist and recreationist, and in which roads, trails, lookouts, and other improvements are necessary.

All too often, foresters overlook the fact that the forest is a biological unit; a unit composed not alone of tree growth but consisting also of other vegetation, the soil and its inhabitants both plant and animal, the insects and fungi, its wild life. As a unit, it must also be considered in its relation to human welfare, health, and recreation; its relation to domestic livestock, farm life; to transportation, to the economics of all business, and in the use of its products. It is easy enough to recognize that the problems of the forest are those dealing with trees, but it is sometimes less easy to see the relationship which exists between the trees and the minor forest vegetation, between trees and soil and the soil life, or between trees and wild life.

In the early stages of forestry we were confronted with the task of indicating a silviculture which might be put into practice immediately. Because of this need, foresters chose the empirical route and have followed this up to the present day. This has given us the answers to many questions, but it has also indicated many problems, the answers to which are essential to a proper understanding of silviculture. To these, however, empirical research can not give the answer. It is therefore necessary to discover through carefully planned, controlled, and detailed work what these answers are. This leads us into the fields of physiology, the physiology of growth of which we know all too little; into the fields of nutrition, of which we know practically nothing; into the fields of reproduction,

from seed formation to tree establishment, concerning which we have only fragmentary knowledge; into the fields of heredity and tree breeding, as yet almost untouched. In the entire subject of the botany of the tree, its growth, its nutrition, its development, its reproduction, we have a vast continent to be explored, a continent that will yield us untold wealth as we are able to exploit it. In our anxiety to put the forest quickly under administration, management, and protection, foresters have overlooked and many are still overlooking the fact that the forest is a growing crop subject to the same general conditions of growth as other crops. We have been intent upon the harvest without paying attention to the basic principles through which the harvest has been derived.

Let us consider for example only one species, the longleaf pine (*Pinus palustris*) of the far South, a tree valuable for its lumber, valuable as a source of pulp, and as a source of turpentine. In the production of the latter, the flow of resin is a physiological process. How is the resin formed, and what can be done to stimulate it? Resin production can be related in part to the weather conditions; but is it related also to soil? If to the soil, is it because of the chemical or physical condition or composition of the soil, or is it because of bacterial soil action? We know that trees of the same diameter, on the same soil, on the same site, and to all intents and purposes alike as to crown area, crown position, density of foliage, etc., vary as much as 200 per cent in the flow of gum when treated the same way. Why? Is the ability of the tree to produce resin freely a transmitted character of the plant contained in the chromosomes of the embryo? Can the resin production per tree be increased through breeding, and can the rate of growth be increased in the same manner?

These are physiological questions, their solution is basic to the management of the tree, but, do foresters generally consider such things? How far the strength of the wood is affected by the soil, by the available nitrates, or by other physiological processes which we might change through our form of management is as yet unknown.

The lower forms of forest life, the brush, the grass, and other herbaceous plants have a far-reaching effect upon the forests and upon all forest life. In places, due to man's general destructiveness, brush or chaparral may take possession of the soil, turning highly productive land into a worthless or semi-worthless natural brush desert, where several centuries may be required to re-establish the forest. One does not need to go as far as California, however, to see such change, for even in many parts of the East, as in the Appalachians, the grass, weeds, and other brush or worthless species come in so quickly on the worn out and abandoned farmlands that they make it well nigh impossible for the more desirable tree species to become established, and the planted trees must necessarily compete with an established and well developed sod and brush cover. Similarly, the heavy stands of bunch grass which quickly develop after the removal of the old forest in the Southwest prevent the quick and ready re-establishment of the forest. Planting has in some regions been a total failure because of the intense competition of the minor vegetation for the scanty soil moisture in the growing season. In the East, foresters are even now confronted

with the necessity of eliminating one group of forest plants because they serve as a primary host for the virulent white pine blister rust.

Through the utilization of the minor forest vegetation or the forest range, either by domestic livestock or by game, the volume of forage is reduced and hence the danger and severity of forest fires is materially lessened. However, under extreme conditions, such utilization may even take the young trees along with the grasses and so prevent the reproduction of desirable forest tree species. In parts of the East, and particularly in the hardwood farm woodlot regions, grazing may even change the composition of the forest, eliminate species, compact the soil, start erosion, and reduce the growth rate.

Any forest research which omits the ecological and the physiological factors connected with the growth of minor forest vegetation, even if it considers it for the trees alone, cannot be effective for long, as all forms of plant life spring from the same source and nearly all compete with one another in the struggle for ultimate control. All plant factors must be studied, together with the ultimate objective in mind that all the work leads towards a better knowledge and a solution of the timber growing problem.

All wild life, and possibly also to some extent that form of life which exists either entirely or only in one phase of its existence in the streams and lakes, plays a part in the biological complex of the forest, and for that reason we can not ignore any far-reaching interrelationship which affects to greater or lesser degree both the growing of timber and the perpetuation of wild life. More and more state forest departments are being confronted with the problem of what to do with the fish and game management, and more and more the state fish and game departments are being confronted with forest management problems. The lessons of Europe are that forests and fish and game go hand in hand. Ultimately they will also become more closely related in this country.

It is impossible to separate forest management from wild life management for the two are so hopelessly interrelated that the destruction of one encompasses the destruction, sooner or later, of the other. Many of these relationships are decidedly important from a silvicultural standpoint. In the Southwest the destruction of the mountain lion and coyote has resulted in increasing the number of porcupines to such an extent that the scanty reproduction following cutting and the advance growth, even when in the polewood stage, is menaced by the porcupine, so that something must be done to control the latter if forests are to be grown successfully. In the lake states the wholesale destruction of such carnivorous animals as the fox and the wolf has resulted in an enormous increase of rodents, particularly the rabbit, preventing successful forest planting in many areas. Hunting, trapping, as well as indiscriminate killing of all forms of wild life has resulted in a marked increase in the number of rodents almost everywhere and this may be responsible in large part for the failure of direct seeding as a measure of forest regeneration. According to recent investigations at the Harvard Forest, the elimination of the chestnut in New England has been responsible for a change in the food habits of the common red squirrel to such

an extent that it now destroys every winter large quantities of the buds and twigs of planted trees.

Birds and sheep are well known to be the carriers of such seeds as the juniper and cedar, and other forest-inhabiting species play a part in dissemination and planting of seeds. It is not an uncommon sight in the west to see bunches of small seedlings come up from a squirrel cache where some overzealous chipmunk or squirrel has stored from 5 to 50 seeds in a single cache, and, either through forgetfulness as to location or early germination of the seed, the rodent has lost his food store.

How closely fish and the forests are related is not known as yet. We do know, however, that in the waters which have become so muddied and sullied by forest activities, through erosion, through improper location of forest or wood-using industries, that fish have been eliminated, and streams once famous for their fishing are now so sullied that fish life is practically extinct. We also know that fish prey upon a large number of insects, particularly those whose larvae are aquatic, possibly some of which are of benefit to the forests, and probably some of undoubted detriment. So far, biologists have not considered the problem, entomologists have not, and foresters have not.

The close relationship existing between fungi and the forest is recognized now by everyone. Fungi determine for certain species the rotation age before which the forest must be cut to obtain high quality wood free of decay. Many of our trees are easily wounded by fire and when the wood is exposed, such wounds permit the entrance of fungi which materially reduce the volume of merchantable wood. The chestnut blight has wiped out from our eastern forests one of its most valuable hardwoods, and still another fungus is threatening the existence of the white pine. Because of the readiness and ease with which fungous spores are disseminated through the air, and the long distances which they may travel, who can say when the Douglas fir will not become a prey to the Douglas fir canker? Similarly, who can say when some other of our major and important tree species may not be threatened by some disease at present unknown or inconspicuous in its native locality. Most certainly every forest must be studied from a pathological standpoint.

Similarly, foresters cannot overlook the entomological problems. For years the forester, with the help of the entomologist, has been combating the bark beetles of the genus *Dendroctonus*. In past years, and not so far back either, millions of feet of timber have been destroyed and the forest on thousands of acres has been practically wiped out through the ravages of these scourges of the forest. The destruction wrought by the bud worm in the spruce forests of the North has caused the pulp men to view its ravages with alarm. Lumbering and fire, which together have eliminated other species and caused an increase in the amount of jack pine in the Lake States region, have made conditions much more favorable for the jack pine sawfly. Formerly an inconspicuous insect causing little damage, its rapid multiplication appears to be directly due to the change in forest composition following cutting, which increased its food supply and possibly reduced the number of predatory birds and mammals. Now there is a

wholesale destruction of this increasingly important forest tree. Possibly the increased production of white pine in pure stands has resulted in the increasing damage by the white pine weevil.

Equally important relationships exist between insects and forest reproduction. Many forest trees, more particularly the hardwoods, depend largely upon insects for pollination. After the seed has been produced in the fruit, other insects may destroy the living embryo and prevent its germination. In the seedling stage it is still subject to insect attacks both upon the roots and upon the top.

As with plant diseases, so insects are causing us, as they have caused the foresters of Europe, to turn more and more from the pure stands to the mixed forest as a better protection against their destructiveness.

One might continue and show the relationship of the forest to the soil, both from a physical and a chemical standpoint, and to all forms of soil life, bacteria, protozoa, mycorrhizal fungi, to soil acidity, and the relationship of the forest to light, but it can be realized without going into details, how great an effect these factors may have and do have upon the forest. Yet so far their fields have been barely scratched; there is a great need for a correlation and coordination of forest research—in management, in physiology, in ecology, in pathology, in entomology, in biology, in soils, even in the realm of the meteorology of the forest. Any research as the basis for the management of any one form of forest life, whether plant or animal, that would ignore or leave out of consideration any one of the environmental factors, or any combination of factors whether plant or animal, animate or inanimate, organic or inorganic, could not be certain of obtaining the entire answer. The interrelationship and unity of all biological factors in the forest do not permit an upsetting of the delicate balances between plants and animals, between plants and plants, or between animals and animals. Man is the controlling agent, and all the adjustments to man's need and management should be made with conscious thought, and this conscious thought must be intelligently developed and applied, not from the forest standpoint alone, not from the viewpoint of other life forms alone, but from the biological standpoint, and for this purpose it is essential that all forest research be considered from the biological standpoint, not from that of the wood product alone, and as part of one great problem. This interrelationship and unity should be recognized by all in so far as resources in men and funds will permit in the organization of research in forestry and in the formation of any program which may deal with growing and protecting the forest crop and in its relationship to other factors. This biological attack and study of the interrelationship of all factors is the basis of the organization now in progress for the Federal Forest Experiment Stations.

This leads naturally into the next big problem looming up at the present time, a research personnel. This question is one which has a direct bearing upon all research activities, and is in many ways the biggest problem facing the proper development of forest research today.

The early development of the local forest experiment stations and the amount

of general study and empirical investigations demanded of the field force did much to develop men. Then, too, an apprenticeship for indefinite periods of from a few months up to as high as 3 years at the forest experiment stations resulted in an early weeding out of misfits and of determining the value of the others, and so assisted in the development of a personnel which has been heavily drawn upon in building up the present station force. However, this reserve has now been depleted to such an extent that there is no longer a surplus available.

Up until the last 5 years there has been little incentive for men to spend the time and money required for advanced training in any of the many phases of forest research. There has been little or no assurance of employment in which such training could be used, and there have been practically no fellowships available to aid men without the necessary means. The relatively rapid expansion of silvical research since 1920 has brought about an acute problem in the lack of enough suitably trained men for this most important phase of our forest activity. The result has been that it has frequently been necessary to employ the best men available even though they may not have had that fundamental, advanced, and specialized training which is so desirable. In the forest experiment stations outstanding men from the administrative organization of the Forest Service and the best men from the graduating classes in the forest schools as obtained from the Civil Service registers have regularly and systematically been selected.

While a considerable part of the research of the past decade or more has been of a preliminary character, that of the future must go necessarily far more deeply into fundamental questions. In the early organization and extending even up to the present time, we have been confronted with the fact of obtaining for the timberland owner information which he can readily use now in order to put his lands into a productive state. To this end, as already stated, the stations have had to do much empirical work, far too much empirical work for the ultimate and basic foundation upon which our scientific forestry must rest. With the necessity for undertaking gradually a greater proportion of fundamental work, it will be necessary, more and more, to have men of exceptional ability and training. Those who have already gained experience in research and who have been selected carefully to begin with should be afforded every opportunity for advanced training. The possibilities for training on the job for the right kind of men when under competent supervision are sometimes badly underrated, but regardless of all this, schools and other organizations should systematically encourage and assist their men in getting advanced training. The Forest Service has endeavored to do this, and each year the Forest Experiment Stations are seeing more and more of their men taking advanced work.

The situation, however, already demands a substantial increase in the number of available fellowships as a means of stimulating training. Silvical research in schools, states, and the national government can absorb now each year a number of men with advanced training, so that as a minimum at least 10 fellowships should be available, fellowships sufficiently large to make it possible for men to give all thought and energy to the task, without having to worry

over finances. The fellowships should be available only to the best men and should be available only at the strongest universities and should not be so combined with teaching that their effect is minimized. Already the stage has been reached where there is a critical problem of obtaining men of strong mentality with a training suitable for the most fundamental work, and the demands of the future along this line will undoubtedly become more and more insistent.

In the training of men for forest research, no matter whether they are to be employed in the field of physiology, biology, entomology, zoology, pathology, soils, or silviculture, there should be a good general training and grounding in forestry. Only such a training will give the necessary background for that well-balanced silvical research which will keep the chief objectives of timber growing and protection in mind and prevent specialties from becoming objectives in themselves. Unfortunately, at the present time there are all too few such men available. There are physiologists, ecologists, biologists, soil scientists, and others galore who might be suitable for forest work but most of these have not had that basic forestry training which will assist them in keeping their feet close on the ground where the work really lies.

The schools have a joint problem with the research agencies to produce men of the caliber required. At the present time, there is no one school outstanding in this regard, and furthermore, there are few schools where a man may procure that basic foundation to fit him best for forest research. Some of our forest schools give some training in research, but there is a great need of a school which would make a distinct and definite effort to produce adequately and thoroughly trained men. I sometimes wonder if there is not too much emphasis given to cramming the student with information on forestry and too little to basic principles; I wonder if too many elective opportunities are not given the immature student, permitting him to dissipate his energies in miscellaneous courses, and if there is not too little emphasis upon plant physiology, ecology, soils, or biology. Perhaps this pessimism is unfounded, but in the search for men there are found those who do not or can not distinguish between transpiration and evaporation; who do not know what are the principal factors of site; who are really unacquainted with the facts as to how trees really grow. Can research material be made of such persons?

It is true that the question of statistics is new, the place of statistical methods is not yet fully known, the possibilities of the statistical attack on forestry problems is still largely in the future—yet already we know that there is a place for statistical analysis in forestry. Do we find foresters desirous of entering research with a knowledge of forest biometry?

This national situation calls for attention. To live up to their obligations, the schools should furnish adequately and thoroughly trained men for all branches of forestry. Research should lead the forest profession in every line of work, for research first determines the facts upon which management rests, and research cannot lead, cannot furnish the basic facts, if the personnel is lacking.

Where do the forest students come from? Why do men enter forestry? Who encourages and stimulates them to take forestry as a life work? I have just recently completed a little study of 200 men, graduates of forest schools since 1920, men whom I have personally met and interviewed. Of the 200, 88 per cent stated that the romantic life of a forester appealed to them; that they had read of the wild, care-free life of a forest ranger, that the movie had showed the foresters as stalwart sons of the open, that they loved the out-of-doors, the simple life, they enjoyed camping, they liked to hunt and fish, and so on. The romantic side was awakened by the appeal to nature in nearly every case. Only 13 boys out of the 200 became foresters because they had read of forestry and knew a little of what it meant, but even in the 13, the romance of the forester's life was an evident thing. Three boys went into it for their health, but even here romance entered. But while the romantic appeal may arouse a number of boys, it is particularly significant that those who really found out what forestry was before they made the final decision to enter it, who made real inquiries into the future of forestry, are those men who so far have given the most promise of success. Will we who are looking for the bright, capable, hard-working boy, have to depend upon mere accident and the romantic appeal to get the best men into forestry? To what extent can forest research continue to look to the forest schools for the highest type of men if no effort is made to attract the high type of man to the school? Must accident and chance tell the story? It is not so in many other professions. How long will it continue in forestry?

Much of what has already been said has indicated the fourth problem: how the correlation between the various biological factors can be brought about. What has been said has also indicated the answer to the problem.

Given adequate personnel at a station, given adequate equipment and means, the fact that the research is all directed towards a common biological end will bring about correlation. It will require leadership in the stations, cooperation and help of the personnel, and the concept that the forest is a biological unit. When we really study the forest from the standpoint of its biology, and take into account the fact that it is a unit in itself, made up of a vast number of parts, then and then only will there be a real solution to timber growing and protection.

THE SCIENTIFIC FOUNDATION OF FORESTRY

As Exemplified Chiefly by Forest Research Work in Finland[1]

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I had the honor to receive in December last a letter from the Committee appointed for the International Congress of Plant Sciences, inviting me to lecture before the Forestry Section on the subject "The Scientific Foundation of Forestry as Exemplified by Forest Experiment Station Work."

In setting out to deal with the suggested theme, I may remark that I have been compelled to abridge and to reshape to some extent the subject set me. The fact is that my present duties, which are chiefly of an administrative and practical nature, have prevented me from following the work of the forest research institutions in different countries with that attention to detail which would enable me at this moment to deal with all the main lines taken by such research. In abridging the theme of my paper, I will therefore, with your permission, restrict my remarks, for the sake of greater concreteness, chiefly to the work done in Finland in the field of forest research and to the results obtained there. As far as possible, I shall attempt, however, to keep on the whole to those matters which, *mutatis mutandis*, might be regarded as possessing a special significance also for North American forestry.

In point of fact, between Finland and North Europe in general, on the one hand, and North America on the other, certain notable similarities can be said to exist. A similar so-called Fenno-Scandian climate to that of Finland and Scandinavia prevails over immense areas in the interior of Alaska, the interior of British Columbia, Alberta, and the Rocky Mountains of the United States, in part right down to the Mexican frontier, though naturally at an increased altitude the farther southward we proceed. A similar climate, generally perhaps somewhat moister, also prevails over wide areas in Northeast Canada and the mountainous districts farthest northeast in the United States. Just as in Fenno-Scandia, glacial moraines, eskers, and sands hide the basic rock, so, in North America we find glacial formations over wide areas. The swamps, too, which in Fenno-Scandia are so numerous that in Finland, for example, they comprise 35.7 per cent of the total land-area, appear in great number and extensive form especially in Canada. North American forestry is as little tied to old traditions and the rigid forms of old-established management as that of Finland, so that in each case the future of the forest can be planned with comparative freedom,

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 17, 1926.

but in each case, alike in North America and North Europe, it is obviously of the utmost present importance that *forestry be planned on the most rational lines on the basis of sustained yield.*

Among the investigations carried out in Finland, the materialization of which would appear to possess a significance also for the planning of North American forestry—to begin with, perhaps, in one state, or a few states only—I would mention the recently completed *survey by lines of the total forests of Finland,* the chief features of which are possibly familiar to members of this Congress. Finland has certainly—except for a few of the most northern districts and the Petsamo area obtained from Russia at the Peace of Dorpat in 1920—been mapped throughout by the survey authorities, but as the surveys have been carried out during different periods and supplemented at different times, and the boundaries between forest, swamp, meadow, and ploughland have had ample time to alter considerably during the course of one and a half centuries, any inventory of the Finnish forests based on survey maps was doomed beforehand to unreliability. On the other hand, a detailed inventory of this nature over the whole country would have been much too expensive. In the circumstances, recourse was had to what is called surveying by lines, a method much applied of old in North European countries, and used, for example, in the mapping, during the past fifty years, of the State forests in north Finland. The survey by lines of the total forests of Finland was made in such fashion that the lines, placed at regular intervals of 26 kilometers, were drawn beforehand on a map of the country, in a direction at right angles to the main ridges and the main trend of the waterways. On each line a complete investigation—comprising soil, productivity class (forest-type), species, age of stand, height, standing crop, growth, silvicultural state of forest, etc.—was made of the stands met with along the line, each stand separately on its own blank form, together with a measurement of the length of survey line occupied by the stand; corresponding measures were resorted to for swamps, meadows, and ploughland.

To keep down expenses, actual measurements of trees were not made all along the line; ocular estimates were made of all the stands met with, and at regular intervals, at the end of each second kilometer, a sample plot of 50 by 10 meters was taken for measuring the standing crop [cubic meters per hectare] and growth, and a plot 100 by 10 meters for measurements of merchantable timber (over 20 cm. at breast height). Where a sample plot would have comprised the boundary areas of two separate stands, it was automatically transferred to the stand with the longest length of line within its area. As these sample plots were invariably subjected to previous ocular estimation, it was possible, by using a method of calculation specially devised for the purpose to calculate the correlation between the estimated volume per hectare and the results of actual measurement, and thus to check ocular estimation all along the line; further, the sample plots provided in themselves a wealth of valuable control material for the ocular estimation statistics, checked in this way—the total number of sample plots rising to 4,919.

In addition to its comparative cheapness, the method used in this case has the inestimable advantage over, say, the kind of survey in which every tree along a line of, for example, 10 meters breadth is measured, in that the survey of fairly extensive areas can be completed by its use in a comparatively short time; the method has also greater accuracy to commend it, as a plot of 50 by 10 meters or 100 by 10 meters can be much more exactly defined than a sample plot 10 meters broad with an actual length equal to that of the whole line; it must be remembered that the standing crop of the sample plot is decisively affected by the exactitude with which the sample plot is defined; in other words, by the measure in which trees growing on the boundary are included in the plot. As in the method used, the part of the line, falling within each stand, swamp, etc., had been exactly measured in a longitudinal direction, and all the necessary calculations for each stand, etc., registered, it was an easy task to calculate, from the resulting statistical material of over 100,000 memorandum forms, the percentages of the various classes of land (forest, swamp, meadow, ploughland, etc.), the percentages of different soils, the percentages, as regards forest, of the different forest-types, the different species of trees, the different age-classes and in these, the stands of varying volumes, the percentages of different silvicultural state (state of nature, cared-for, devastated, etc.), and other ratios for the whole country, for the various provinces, the various water systems, phytogeographical areas, etc., and as the superficial measurements of all such areas are given in geodetic maps, the corresponding ratios could be calculated also in absolute measurements.

The outdoor work was chiefly done during two summers, and the cost worked out at 0.00043 dollars per hectare of land-area in the whole country. Mathematical-statistical calculations and control measurements over smaller areas along lines set closer together showed the spacing of the lines to have been adequate; a more closely-set system of lines would only have considerably raised the cost, without appreciably affecting the exactitude of the results. If the area to be inventoried is larger than Finland (34.4 million hectares) and larger sub-areas will suffice, the lines can of course be placed farther away from each other in proportion, with a corresponding decrease in costs. This kind of careful inventory of forest resources and the state of forests is obviously of extreme importance. It provides a reliable foundation on which the whole forest policy of a country in its most important aspects may be based.

To procure the full benefit of a survey by lines of this nature, which must naturally *be carried out in so brief a time that the material is in its entirety* (*of a homogeneous nature as regards age*) *either in respect of the whole country or the area selected for survey*, certain *preliminary measures* are essential. By these I do not, naturally, intend so much the checking and the working-out of the methods used in measuring sample plots and individual trees, for in this respect the methods in general use provide, on the whole, sufficient guarantee. Nor do I intend the methods by which, on a mathematical-statistical basis, ocular estimates are verified, or the general working-out and application of mathematical-statistical methods to surveying by lines, as in this respect there are already in

existence fairly reliable and exact methods, including some methods worked out quite recently in Finland. I intend above all the creation of an objective principle of classification according to quality.

The necessity, from many points of view, for *classifying sites on the basis of forestry* is well known. The productivity of different sites varies so greatly that no calculations as regards yield and profitability can be arrived at before the conditions of productivity classes of the area in question have been elucidated. A classification of this nature is equally essential from the silvicultural point of view, as the choice of species is decisively affected by the quality of the site, and even with the use of the same species the silviculture on sites of different quality will be totally different. For the various productivity classes to correspond from the point of view of mensuration, it is naturally not sufficient, however, as it is customary to do, to determine productivity classes separately for each species of tree. The forest lands of a given area must be capable of being classified according to quality irrespective of the species of tree grown, thus making it possible, for example, supposing the whole area in question were to be cut down, or a change made in general in the proportion of different species, to procure for each site the species of tree which in the prevailing conditions would be most profitable for that particular quality of site. In other words, in drawing up, for instance, yield tables, a *common classification according to site quality should be applied to all species of trees*, in such manner that a certain grade in respect of 1 tree corresponds exactly to the same grade for a second, a third, a fourth, etc., species, as only on this condition is it possible to make comparative calculations as to the profitability of different species on any site, the importance of this common classification increasing with the number of different species grown in a country. Obviously also, the *classification for mensuration purposes should be identical with that for purposes of silviculture*, so that the use of different qualities of site for the same species, depending on whether silvicultural or mensuration considerations have been allowed to rule, is avoided.

The only classification that fulfils all these conditions is a biological one—that is, *sites must be classified for purposes of forestry in such manner that sites in the main of the same value in a biological sense are entered in one class, and those of different value in different classes.* In Finland, as may be known, the means used for obtaining such a classification of productivity has been the division into so-called *forest types.* In this case all those stands are referred to the same forest type the vegetation of which at or near the time of maturity of the stands and provided the stands are normally stocked, is characterized by a more or less identical floristic composition and by an identical ecologico-biological nature, as well as all those stands the vegetation of which differs from that defined above only in those respects which (being expressions of differences due to age, fellings, etc.) have to be regarded as merely accidental and ephemeral, or at any rate as only temporary. Permanent differences call forth a new forest type in cases where they are sufficiently well marked, or a sub-type in cases where they are less essential, but, nevertheless, noticeable.

The investigations of Yrjö Ilvessalo (1920) have shown that the goal aimed

at is really attained as regards mensuration, and those of Lönnroth (1925, 1926) have in a brilliant manner confirmed this result. The investigations of Valmari (1921) and Aaltonen (1925, 1926) have supported these results from the standpoint of the science of soils, and those of Linkola (1924) from the point of view of plant biology, while silvicultural investigations, too, have led to the same general results. For the survey by lines of the forests of a country it is in every case essential that the principle of classification of sites according to quality is defined beforehand as exactly and as objectively as possible. It is further of extreme importance that in drawing up growth and productivity tables for the chief tree-species of a country, the same principle of classification is used as in the survey by lines, in which case it will be possible, amongst other things, to estimate with full objectivity the extent to which the productivity of the country's forests can be raised by growing on each site the species most profitable on that site, and by expending on this species the care demanded by it.

In areas where swamps prevail to any considerable extent, it is naturally not enough, even from the point of view of forestry, to restrict attention to the forests, but the swamps too must be investigated. An appreciable proportion of swamps are such that when drained they can be made to produce fully satisfactory forest. Of the Finnish swamps, for instance, about 40 per cent are drainable for forestry purposes. In the measure in which the forest area diminishes, particularly as land is brought under the plough, it will be necessary to have recourse to the reserve of forest land represented by the swamps and to begin adding to the area of productive forest by draining these. As, however, the potential afforestation value of different kinds of swamps varies considerably, the necessity for a reliable classification of swamps according to their afforestation capacity becomes apparent. Swamps have been classed in Finland for this purpose, using vegetation as a basis also in this case, into so-called *swamp types*, and as a matter of fact, the investigations of Tanttu (1915) and Multamäki (1924) have shown that after ditching, the various swamp types tend towards definite forest types, becoming finally transformed into these if the ditching has been effectual, in which case they display in the main a growth as good as that of the corresponding forest types on mineral soil. To obtain the full benefit from surveying by lines the principle of classification of swamps from the forestry point of view must be biologically defined beforehand in such manner that it will subsequently be possible to calculate from the survey the amount of swamps available for ditching, and the extent to which the different afforestation-capacity classes are represented in these.

A survey by lines permits, further, of as accurate an inventory being made of meadows, fields, etc., as of forests and swamps, and it is indeed worth while, even in a survey intended to serve purely forestry purposes, to classify at least roughly also fields and meadows, and similarly to classify swamps in respect to their suitability for agricultural ends, from the point of view of the fuel and peat industry, etc. But it is obvious that the labor involved will be heavier and the main purpose suffer in proportion as tasks not directly connected with a forest survey are included.

It is worth mentioning, however, that by following the method of classification according to quality used in Finland, namely, the classification of forest land into forest types and swamps into swamp types, an incidental result of the survey by lines is the attainment of extremely important information from the point of view of colonization policy regarding the *fertility of different* districts. Thus, by calculating for each kilometer of line the percentages of the most productive—or, we might say, the most exacting—forest types, the next best, and so on, one obtains, particularly if attention is paid also to soils (and the degree of stoniness) an exceedingly illuminating, comprehensive, and objective picture of the degrees of fertility of different districts, and consequently of their possibilities for prospective colonization.

From the standpoint of forestry a survey by lines is still not in itself sufficient, but must be supplemented by a study of *forest consumption* in which attention must be given both to the average losses due to accidental causes—forest fires, storms, insects, etc.—and to consumption for domestic and industrial purposes, as well as to possible exports of unworked timber. In order to establish in a fully satisfactory manner the balance between growth and consumption it is essential for the survey by lines and the investigation of consumption to be carried out as nearly as possible simultaneously.

On my own part I am thus prepared to assert that the inventory of forest resources and forest growth by lines provides excellent material for placing a country's forest policy, and in part also its agrarian policy, on a right footing. For it to answer fully its purpose, an inventory of this description calls, however, for important preliminary labors, above all the establishment of the classification of forest and swamp lands according to quality on a coherent and objective basis for the whole area of investigation, and no less for important parallel labors, namely, the drawing-up of growth tables for the chief species of trees, using the same quality-classes for all species. At approximately the same time as forest resources are inventoried, the rate of consumption should be investigated, to permit of the striking of a balance between growth and consumption, for the whole country and for the various districts separately.

I will now beg your permission to deal more closely with questions appertaining to *silviculture*. It cannot be denied that at present there is often too much of a schematic spirit in silviculture, and that the care of forests is arranged in great part to suit preconceived opinions. The different schools may hold sharply diverging views; often one may speak outright of fashions in silviculture. Sooner or later, however, the too schematic care of a forest is bound to revenge itself; one cannot with impunity treat forests in a cut-and-dried manner. It is wrong to ask oneself such questions as the following: Are forests generally to be regenerated by sowing or planting or by natural regeneration? Are forests to be cut in compartments or by selection? Is management to be based on large or small stands? Are forests to be grown pure or mixed? The one alternative will be right in some conditions, the other in others.

The sowing, planting, and cutting methods, etc., now in use, are undoubtedly in themselves suited on the whole to their various purposes. In other words:

the actual methods of forestry are in the main satisfactory as methods; although particularly as regards thinning and selection cuttings, there is without doubt room enough for further development. All the more important, therefore, is it to be able to judge correctly which of the silvicultural methods is the most favorable in the circumstances. Here one cannot allow the "practiced eye" to decide unaided, for it is too often mistaken, and it is probably still more dangerous to leave the decision to the dogmas of the various schools. The *choice* of the right silvicultural method in each *separate case*—and that is obviously the chief aim of practical forestry—demands a comprehensive knowledge of the *life* and *biology* of the forest. The practiced eye must not be allowed to decide blindly, but the decision must be left to a practiced eye schooled both in economical and in biological knowledge. In proceeding I shall touch only upon the latter, the biological side of the matter.

A person entrusted with the care of forests, or who superintends the care of forests, should know how a forest *reacts* to any of the measures applied to it. Such knowledge is, however, as yet only partly available to any one. Before the practiced eye can be adequately educated in this respect, an intensive work of investigation in forest biology must be undertaken. This comprises several aspects: (1) A comprehensive inquiry into the biology of the various species composing the forest; an inquiry into their seed-production in different circumstances, their capacity for throwing off shoots in different circumstances, the rates of growth in different circumstances, their need for light, their capacity to withstand frost, etc.

(2) An inquiry into the biology of the standing crop, separately for each species of any economic significance; an inquiry into their natural thinning and the resulting division of the individual trees into development (canopy) classes and the development of these classes, as regards both their roots and their trunks and crowns; the relation of different species to each other in the same stand, etc.

(3) An inquiry into the biology of the entire vegetation of a stand; into the part played by weed vegetation in regeneration areas in general, and separately for the various species composing it, into the influence of undergrowth on the thriftiness of a forest in its later stages, its influence on the transformation of forest land into swamps, etc.

(4) The significance of destructive fungi and insects under different conditions.

(5) The significance in different conditions of the microbiological flora and fauna of forest land.

Despite the investigations already made, much additional investigation is still needed in the normal climate of stands in different conditions as compared with the general meteorological climate, in the normal formation of humus and the general formation of soil in forests in different circumstances, in the influence of different cutting methods and other silvicultural measures on the climate of a stand and on the normal condition of its soil, and in the consequent reaction of that on the vegetation of a forest as a whole, and separately in the reaction on the development of the standing crop. In all these investigations it is not

sufficient to establish only the correlation between different phenomena, but the investigator must strive at understanding them in their *physiological* or *biological implications*.

It is my firm belief that silviculture is in essential need of this biological foundation, which—need we deny it—is still, despite the copious investigations already carried out in these fields, only incompletely in existence, and is only to be achieved by the most intensive investigation work. It might perhaps be remarked that the conditions in forestry are still on the whole so extensive in nature that this kind of biological foundation is unnecessary. This I am inclined gravely to doubt. It is before all in *extensive* conditions that results have to be achieved with the *minimum of expenditure*. And how is this to be possible unless the forester working in such conditions knows how a forest will react to every, even to the least costly, measure? In silviculture, even the profoundest knowledge and inner perception of forest biology is surely not unnecessary, still less a hindrance, provided of course it has not been attained at the expense of practicality.

We have become accustomed in forest investigation work to rely to a great extent, often to the greatest extent, on experimental methods; indeed, we quite generally term forest research institutions "forest experiment stations". In the field of silviculture we have, however, in my opinion, greatly over-estimated the significance of experiments—owing perhaps in some measure to the close connection with agricultural investigation work. Yet in inductive scientific research there is the *comparative* method to resort to as well as the *experimental*. Forest experiments have the general drawback that owing to slow growth and the high age reached by forests they need extremely long periods to lead to definite results. Against this, fully reliable results can be obtained much more quickly by comparative methods. For forest in its natural state and forest under management contains an unlimited number of objects of investigation ready to hand; the investigator must only know how to choose the most instructive of these for his particular purpose at the time.

Such observation-stands or fractions of stands do not, it is true, fulfill the highest demands in every respect, but the number of them is so great that the effect of disturbing influences can be largely eliminated. The biological investigation of forests in particular should be, quite in a decisive degree, based on comparative research in nature, as it is frequently absolutely impossible to bring about the desired conditions artificially by means of deliberate experiments, and in the latter case the investigator has very often had time to die long before the experiment has reached the required stage. This is by no means to be taken as implying that it is unnecessary to *supplement* and *complete comparative research* by *actual experiments*. Indeed, experiments can be arranged to much more purpose, if the matter in hand has previously been sufficiently elucidated by comparative research; all that I intend is that in my opinion the experimental work should not be placed in the foreground. Thus it is my opinion that silvicultural-biological research in particular should at least for the present be based chiefly on the material held out by natural and commercially managed

forests in themselves. In greater degree than to Finland, this applies, as far as I can see, to North America, where the proportion of natural forests is still so immense.

In comparative research of this nature it is naturally essential that the trees, groups, stands, etc., compared are truly comparable. Here we are faced again with the question of the appropriate classification of afforested sites according to quality, a classification, in effect, in which sites of equal value from a biological standpoint are really grouped together and those of different value kept apart. Whether the subject of research concerns the sparsity or density of growth, the influence of one or another species on, for instance, the formation of humus, or let us say the microflora of the soil, all comparisons and the results derived from these will be found to limp, if the stands concerned are not situated on sites of biologically equal value. The exact classification of sites is essential not only for the objective treatment of comparative observations—or for that matter of experiments—but it is equally necessary for the right utilization of the results. The application of the results in practice presupposes in the applier exact knowledge of the general conditions and in particular of the kind of site on which the results were obtained.

This leads speculation a step further. Many silvicultural textbooks and manuals contain a special department devoted to *applied silviculture.* By this is chiefly intended the care of different species of trees. Nevertheless, the duty of applied or, more rightly, *specialized silviculture,* cannot be merely to give an account of the silvicultural care of different species, because, as already mentioned, even the care of the same species must be arranged in a totally different manner for different sites. Thus, the site is fairly decisively responsible for the choice of the natural regenerative method, the method of thinning, etc., for the nature of eventual growing of standards or growing of underwood, etc. The silviculture of a forest must be arranged to conform with the demands of the site, which leads inevitably to the conclusion that specialized silviculture must, above all, comprise the silviculture of different qualities of site, of biological productivity classes.

The whole of the forest-biological research referred to earlier has thus, in quite a decisive manner, to be directed to the comprehensive elucidation of the life and vital demands of different biological productivity classes. From an extremely theoretical starting-point we have thus arrived at an eminently practical aim, for with the elucidation of the silvicultural care of each of the chief biological productivity classes, we shall be in a position to give the practical forester exceedingly concrete directions for different eventualities. It means—if the lines staked out by forest research in Finland are followed—that what has to be worked out is above all the *silvicultural care of different forest types,* which would thus form the chief subject of applied silviculture.

Passing by many other important fields of research, such as the question of the cultivation of tree-species outside of their natural areas of distribution, of the geographical species of trees, the deterioration of forest land into swamps and its prevention, the afforestation of swamps and open lands in general, and

many others, I beg your permission to refer, before concluding my paper, to a certain formal aspect of forest research. As in biological research in general, the results arrived at in forest research work are not as a rule as absolute as they can be, for instance, in chemistry or physics, the degree of variation in the properties of the objects to be observed being usually considerably wider. The results are mostly in the nature of an average struck from numerous more or less variable observations or measurements. In this case there is seldom recourse to exhaustive statistics, and one has generally to be satisfied with measuring or otherwise observing a larger or smaller number of individual cases; in other words, one has to rely on representative statistics. Such being the case, it is of course essential in working with statistical material of this nature to adhere closely to the methods generally approved for representative statistical research, that is to say, a wide use must be made of *mathematical statistics*. This is as essential to the preparation of volume, growth, and yield tables as it is to the treatment of line survey statistics, to the elucidation of the interior construction of a stand and its development, and to the investigation of the vegetation of a stand. Without the checking and control provided by mathematical-statistical research methods, the results of the most careful research work based on representative statistics can be of only doubtful accuracy. And as we all know, for practical purposes the accuracy of the results arrived at in research is a prime consideration.

The science of forestry must serve the practical management of forests. It must be capable of providing practical forest management with the most practical weapons in an easily utilized form. Yet this does not imply that the methods of research should also be simple and easy to apply. From the history of silviculture one could quote exceedingly illuminating examples of the application of "practical methods" to research work, or in other words, of attempts that have been made to investigate complicated matters with inadequate preparation and insufficiently exact and reliable means of investigation, with the result that the outcome of such research was of as little practical use as of scientific value. For a person using a telephone it is altogether immaterial to know how complicated were the processes determining the invention of the telephone; for him the main thing is that the telephone he may chance to use or own works properly and that it is easy to care for and to use. Similarly, it is nothing to the forester engaged in practical work how laboriously any of the results of the science of forestry have been attained; the chief thing for him is that the result is reliable and that it can be applied without difficulty to practice. In spite of the fact that forestry is an *eminently practical field*, the worker in the science of forestry may not draw back even from the most difficult and complicated *methods of research* if reliable and practical results are not to be achieved in any other way. The guiding principle of scientific forest research might be formulated: *scientifically valid methods of research but purely practical aims.*

THINNING AND INCREMENT[1]

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If a natural stand of spruce, for example, is supposed to start with about 5,000 seedlings per hectare, experience shows that the number of stems will be reduced by and by as the trees grow larger. At 100 years of age there are not so many as 500 trees left per hectare, even if no cutting has been done in the stand. The basal area at breast height in a forest stand reaches a figure amounting to less than 1 per cent of the area on the ground occupied by the trees, and as a rule we cannot get more than about 50 square meters basal area on 10,000 square meters of forest land, which means only $\frac{1}{2}$ of one per cent. When the forest is growing too densely a lot of stems will be choked to death.

The original purpose of thinning operations was to facilitate this reduction of the number of stems by taking out in good time such stems as were going to die. However, a remarkable development has taken place, and the modern principle of thinning is not limited to the burial of the dead, but pretends also to regulate the composition and the increment of the stand for the future.

The trees remaining after a thinning will grow better than before, so it is possible even with heavy thinnings to get the same amount of increment on the reduced wood capital as was produced before. There are also experiments showing an increase of production when the cubic volume of the stand is reduced. These figures, however, are not quite certain on account of the difficulty of accurate estimation. The question cannot be settled definitely without the use of comparative experiments, and no 2 sample plots are ever exactly equal from the beginning. The small differences in the figures for total production are, accordingly, often dependent upon other conditions than thinning.

Much more important is the following question: To what degree is it possible to carry out the operation of thinning without lowering production? If in a certain case we can get the same amount of increment with a standing volume that is $\frac{2}{3}$ of what is usual, that reduction of the capital will give us an increment per cent that is $\frac{3}{2}$ times the former per cent, and if the capital could be reduced to $\frac{1}{2}$ that before thinning, the rate of interest would be doubled.

Besides increasing the per cent, the heavy thinnings have another effect of economic importance. The increment will be placed upon the best trees and the

[1] Presented before the International Congress of Plant Sciences, Section of Forestry, Ithaca, New York, Aug. 20, 1926.

quality of the wood will be raised at the same time, since large dimensions are sooner obtained. Thus the final product will be more valuable and the heavy thinnings give good revenue from the stand at an earlier age, which considerably influences the economic results.

Fig. 1. Sample plot 54, Section II, Dalby Crown Forest, Skåne, South Sweden. Spruce forest plantation. Ground cover of needles and twigs. Light, low thinning.

In order to illustrate the effect of heavy thinnings I have chosen the experimental series No. 54, consisting of 4 sample plots, located at Dalby in Skåne, in southernmost Sweden. The plots—the area of each being 0.25 hectare—were laid out in an even-aged spruce forest, 31 years old in 1906. The man who started this experiment was the first chief of the Swedish Institute of Experimental Forestry, A. Maass. From 1909 to 1925 the late Professor G. Schotte carried out the thinnings, and in 1922 he made a preliminary report of the results. Professor H. Hesselman has carried out investigations of the flora and the soil, and the results were published in 1926 in the report of the Institute (Hesselman, Henrik, "Studien über die Humusdecke des Nadelwaldes," etc.). In 1926 the plots were thinned and estimated for the fifth time, so that the Swedish Institute of Experimental Forestry has had them under observation during 20 years. In April, 1926, when the author of this article made the thinnings, special measurements were carried out with a transit in order to check the height curves, and comparison was made between the curves from the different estimations. The figures therefore ought to be as correct as possible.

Section I has been left untouched all the time, except for dry stems, which have been removed. Section II has received moderate, low thinning, and only suppressed and dry stems have been cut away. Section III has been given heavy, low thinning and Section IV extra heavy thinning (from below). Of the total wood production until now, at 51 years of age, 51 per cent has been taken out by thinning in Section IV; 40 per cent in Section III; 22 per cent in Section II; and 8 per cent in Section I. The method of thinning in Section IV, however, was not extra heavy from the beginning in 1906, but only heavy. In 1916 the cutting was changed to the extra heavy degree, and the three last thinnings have been typical of that method. For the calculations of the economic effect this matter is of importance.

In 1920 the intensity of light was measured at breast height in the different sections and comparison made with the intensity of light in an open field. It was found that in Section I the intensity of light was only 2 per cent of that in the open field. For Section III the corresponding figure was 5 per cent, and for Section IV, 9 per cent. Section II was not investigated at all in this respect.

The soil is now a sandy "Braunerde" type on moraine, and when the stand was planted the vegetation belonged to the *Calluna* type. Under the 46-year-old spruce forest in 1926 there was practically no vegetation at all on Section I and very little on Section II. On the 2 other sections the ground vegetation was examined with the aid of quadrats of 0.5 square meter area, laid out at a regular distance of 9 by 9 meters on which all plants were counted. Some figures, illustrating the difference in the vegetation on the two investigated sections, are noted in Table 1 below.

TABLE 1. FREQUENCY OF SOME PLANTS ON THE FOREST FLOOR. EXPERIMENTAL SERIES 54

Plant	Frequency per cent	
	Section III	Section IV
Viola silvestris	1	43
Gramineae	–	31
Campanula rotundifolia	3	35
Rubus idaeus	3	23
Veronica officinalis	6	20
Sorbus Aucuparia, seedlings	6	7
Picea excelsa, seedlings	22	95

In addition to the plants given in Table 1, it may be mentioned that seedlings of several other trees appear on both sections and that several herbs, such as *Lotus*, *Cirsium*, *Epilobium*, *Fragaria*, etc., have been found on Section IV only.

The differences in ground vegetation show very clearly that the extra heavy method of thinning has produced a much more marked reaction in the humus covering.

The figures per hectare of the number of stems, basal area, and cubic volume on the different sections at the beginning of the experiments, before the first thinning at 31 years of age in 1906, are given in Table 2. The cubic volume is calculated as stem wood with bark. The basal area is also computed with bark.

TABLE 2. THE STAND BEFORE THINNING IN 1906

Section	Number of stems per hect.	Basal area in sq. m. at breast height per hect.	Volume in cu. m. per hect.
I	6,040	45.5	266.7
II	4,724	43.0	255.5
III	5,772	46.4	273.6
IV	4,476	43.3	258.9

Section III was the best developed part of the stand, the quality of site being a little higher. Sections II and IV were almost equal. It may, therefore, be especially interesting to compare the differences in development on these two sections, caused by differences in treatment. Sections I and III are also very nearly equal, Section III being a little better.

The average of Sections I and II, compared with the average of the two other sections will show figures that are very nearly the same (Table 3).

TABLE 3. THE STAND BEFORE THINNING IN 1906

Sections	No. of stems per hect.	Basal area in sq. m. at breast height per hect.	Volume in cu. m. per hect.
I, II	5,382	44.3	261.1
III, IV	5,124	44.9	266.3

In Sections III and IV the number of stems is 5 per cent lower, the basal area at breast height is 1 per cent larger, and the volume is about 2 per cent greater than in Sections I and II. It is impossible to avoid such small variations in forest stands, and the groups therefore must be said to be comparable to a very high degree.

The last estimation in 1926 gave the figures presented in Table 4.

TABLE 4. THE STAND AFTER THINNING IN 1926

Section	Thinning method	No. of stems per hect.	Basal area at breast height sq. m. per hect.	Volume in cu. m. per hect.
I	Cleaning	2,636	59.2	585.0
II	Moderate thinning	1,744	48.7	489.1
III	Heavy thinning	1,232	39.8	407.6
IV	Extra heavy thinning	812	32.0	320.5

The difference as regards the degree of thinning can be pointed out by observing that Sections II and IV now show a difference in volume of about 170 cubic meters per hectare, while from the beginning they were practically exactly equal. The number of stems on the extra heavily thinned plot is now, at 51 years of age, less than half that on the moderately thinned one. The untouched Section I has now a volume exceeding that of Section III by almost 180 cubic meters per hectare.

The figures of how many cubic meters have been taken out by thinning at different times will be seen in Table 5 below.

TABLE 5. THINNINGS MADE ON THE DIFFERENT SECTIONS

Year	Section I		Section II		Section III		Section IV	
	Cu. m. per hectare		Cu. m. per hectare		Cu. m. per hectare		Cu. m. per hectare	
	Thinning	Remaining	Thinning	Remaining	Thinning	Remaining	Thinning	Remaining
1906	12.5	254.3	14.2	241.3	42.1	231.5	29.2	229.8
1911	8.8	375.4	13.0	352.4	31.2	333.8	20.5	337.2

We shall now proceed to study the figures of increment and total production. At first we will take a glance at the periodic volume increment, demonstrated in Table 6. The figures for per cent are calculated with the use of compound interest.

TABLE 6. CURRENT ANNUAL INCREMENT IN VOLUME

	Period years									
	1906–1911		1911–1916		1916–1920		1920–1926		Average	
number	Cu. m. per hect.	Per cent	Cu. m. per hect.	Per cent	Cu. m. per hect.	Per cent	Cu. m. per hect.	Per cent	Cu. m. per hect.	Per cent
I	21.7	7.1	16.2	4.0	16.6	3.5	18.8	3.5	18.6	4.7
II	20.7	7.2	16.5	4.3	15.2	3.6	20.4	4.3	18.5	5.1
III	22.3	8.0	18.7	5.0	17.9	4.6	20.6	5.0	20.1	5.9
IV	21.3	7.5	17.9	4.8	18.0	5.2	20.0	6.0	19.5	6.1

The average annual increment seems to have been increased by the heavy degrees of thinning. The untouched area and the moderately thinned section have given 18.5 cu. m. increment per hectare annually, the two heavily thinned plots about 20 cu. m. It is evident that the small differences of quality have not had so much influence as the degree of thinning.

The figures for the average annual increment of the basal area at breast height during the last twenty years point out the same case still more clearly. These figures are to be found in Table 7.

TABLE 7. AVERAGE CURRENT ANNUAL INCREMENT OF THE BASAL AREA AT BREAST HEIGHT

Section	Annual increment	
	Cubic meters per hectare	Per cent
I	1.2	2.4
II	1.2	2.7
II	1.5	3.4
IV	1.5	3.7

The conclusion must, therefore, be the following: For wood-volume production a very moderate thinning has little influence. The heavy thinnings—40 to 50 per cent of the total wood production being already removed at about 50 years of age—seem to have the effect of increasing the total yield.

But the chief point is, nevertheless, the question of the increment per cent. From Tables 6 and 7 it can be seen that the average annual increment per cent is higher the heavier the thinnings have been carried out. We get an evenly increasing series from 4.7 per cent in the untouched forest to 6.1 per cent in the extra heavily thinned stand.

The figures indicating per cent show very sharply the economy of heavy thinnings. We can get more than 1 per cent higher rate of interest without lowering the production; indeed, it seems even possible to a certain extent to increase the production.

Moreover, it must be noticed that the economic value of the yield from a forest stand is not only dependent on the quantity of wood but also on the quality and on the time when we get the yield. The price of the timber will be higher in a heavily thinned forest on account of the greater dimensions of the trees. And the total money value during a rotation must be much higher, as in this case we get a considerable revenue earlier. The income from a thinning operation ought to be reckoned forward with compound interest if we wish to compare the total value of the product from areas thinned by different methods.

The price differences between larger and smaller dimensions are very variable in different countries and under different conditions. I must therefore neglect this factor here, but we can state that the heavy thinnings would have been much more favored by a calculation based on money values.

The total production of wood to the age of 51 years has been 638 cubic meters per hectare on Section I, 625 cubic meters on Section II, 675 cubic meters on Section III, and 648 cubic meters on Section IV. Dividing the plots into 2 groups we get a total wood production of 632 cubic meters per hectare on I and II, and 662 cubic meters per hectare on III and IV. The group III and IV had at 31 years of age produced 2 per cent more than the other group. At 51 years of age the difference amounts to nearly 5 per cent.

Hitherto we have been dealing with thinnings from the point of view that they develop the stand, probably increase the production of wood, cause higher

rate of interest on our growing capital, and make the final yield more valuable. All these things are of the greatest importance for the future, and in forestry it is necessary to work for the future.

But we are working in the present. And the heavy thinnings give us a continually good revenue from the forest; they make the management pay a return on the investment.

Then the following question arises: How much do we get from the forest by thinning? During the time when the stand is growing up to timber and we are waiting for the final yield, what per cent of the wood capital can we take out annually by thinning? That figure would make it possible to judge better about the advantages or disadvantages of investing capital permanently in forestry.

For the experimental plots discussed in this paper we know how much wood the different sections held on different occasions and how much wood has been taken out by thinning. As the experiment started with the thinning in 1906 and ends with the thinning of 1926, we must also include a 5-year period before 1906.

Reckoning with compound interest for that 25-year period we get the following figures for the cuttings:

Section I. The thinnings, consisting of dead wood only, correspond to an annual yield amounting to 0.5 per cent of the wood capital.

Section II. The thinnings correspond to an annual yield amounting to 1.3 per cent of the wood capital.

Section III. The thinnings correspond to an annual yield amounting to 2.9 per cent of the wood capital.

Section IV. The thinnings correspond to an annual yield amounting to 3.8 per cent of the wood capital.

Summary

In this article the author has made an attempt to treat the problem of wood production from an economic point of view, although the figures do not represent units of money. By computing the volume production and the cutting in cubic meters of wood, and by comparing the average increment per cent on the different plots, it may be possible to get general conclusions about the effect of different degrees of thinning.

There are therefore 4 points to be studied: (1) The average increment during the period under investigation; (2) the average rate of interest (the average increment per cent); (3)[2] the total value in money of the wood produced; (4) the annual cut in per cent of the wood capital during the period of thinning.

The figures from the experimental Series 54 show that the natural thinning of dying trees in the untouched forest during the 25 years (ages 26 to 51 years), in the spruce stand, has been 0.5 per cent annually. The moderate thinning amounts to 1.3 per cent, the heavy thinning to 2.9 per cent, and the extra heavy reaches an annual cut of 3.8 per cent.

[2] The third point has not been discussed in this article on account of the differences between the prices in America and Sweden.

The average increment remains about the same for these very different forms of treatment. The heavier grades of thinning even seem to cause a somewhat greater production.

The total production of wood up to the age of 51 years has been about 5 per cent greater on the 2 heavily thinned plots than on the 2 other sections. Until the age of 31 years—before the thinnings—the 2 plots later heavily thinned had produced 2 per cent more wood than the other sections.

The rate of interest on the growing wood capital has been considerably increased by the heavy types of thinning. Thus the average annual volume increment per cent between the ages of 31 and 51 years has been 6.1 per cent with the extra heavy thinning; 5.9 per cent with the heavy thinning; 5.1 per cent with the moderate thinning; and 4.7 per cent with the cleaning.

The heavy thinnings improve economy in forestry from all points of view. But the work of marking the trees is more difficult than in the case of moderate thinnings, and a well trained personnel is needed for securing good results.

UEBER REZIPROKE BASTARDE[1]

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A. Einleitung

In dem Schwabenlande Württemberg, dessen Universität Tübingen ich hier zu vertreten die Freude habe, wirkten 3 Männer als Pioniere der pflanzlichen Vererbungslehre: Camerarius, Kölreuter und Gärtner. Von diesen haben die beiden letzteren ihre Aufmerksamkeit schon den Problemen zugewandt, denen die folgenden Ausführungen gewidmet sein sollen, d.i. der Frage des Ausfalles reziproker Kreuzungen zwischen denselben Pflanzen.

Beide, Kölreuter wie Gärtner, waren zu dem Ergebnis gekommen, dass beim Wechsel der Stammeltern bei der Bastardbefruchtung, die sie allein als Kreuzung bezeichneten (Gärtner, '49, S. 220), also bei den reziproken Verbindungen, die Produkte einander stets glichen. Kölreuter (1762, S. 45) stellte das zuerst 1760 für die reziproken Verbindungen zwischen *Nicotiana paniculata* und *N. rustica* fest mit den Worten: "Sie sind . . . . in allen Stücken so ähnlich gewesen, als ein Ey dem anderen, so ähnlich, dass ich selbst öfters beiderlei Arten nicht hätte voneinander unterscheiden können, wenn sie nicht an den Nummern zu erkennen gewesen wären." Und Gärtner (S. 222, 223) bezeichnet es als "die wichtigste und interessanteste Erscheinung bei der Kreuzung (also reziproken Verbindung zweier erblicher Formen) die vollkommene Gleichheit der beiderlei Produkte."

Von ihnen übernahm dann Mendel seine Uniformitätsregel, d.i. "die allseitig bestätigte Erfahrung, dass es für die Gestalt der Hybride gleichgültig ist, welche von den Stammformen die Samen- oder Pollenpflanze war."

Dass die Gleichheit reziproker Bastarde indessen nicht allgemein ist, das war ja auf tierischem Gebiet schon längst bekannt und durch zahlreiche, besonders auffallende Beispiele belegt. Dass aber auch auf pflanzlichem Gebiete die Regel der Gleichheit reziproker Verbindungen keine allgemein gültige war, das wussten ebenfalls schon die Väter der pflanzlichen Bastardierungskunde. Besonders auffallende Verschiedenheiten bezüglich Gestalt und Farbe der reziproken Verbindungen zwischen *Digitalis obscura* und *D. lutea* fand z. B. schon Kölreuter, und Gärtner stellte solche Ausnahmen von seiner Regel bei verschiedenen Arten fest.

Seitdem sind, wie wir alsbald näher betrachten wollen, noch reziprok verschiedene Bastarde in sehr verschiedenen Verwandtschaftskreisen festgestellt

[1] Presented before the International Congress of Plant Sciences, Section of Genetics, Ithaca, New York, Aug. 17, 1926.

worden, wenn sie auch immer relativ selten blieben, gegenüber den reziprok Gleichen.

Nach den Mendelschen Grundregeln in ihrer Verbindung mit der Chromosomentheorie erscheint nun aber die Gleichheit der reziproken Bastarde nicht überraschend, im Gegenteil, auf ihrem Boden wird *die Verschiedenheit reziproker Verbindungen zum Problem.*

Diesen Problemen nachzugehen, soll die Aufgabe der folgenden Ausführungen sein.

Bei der Darstellung dieser Fragen wird es nötig, uns eine gewisse Beschränkung aufzuerlegen. Einmal sollen die Betrachtungen, abgesehen von gewissen Hinweisen auf tierische Objekte, auf das Pflanzenreich beschränkt sein; z. a. aber soll von Erörterungen der Möglichkeit oder Unmöglichkeit reziproker Verbindungen hier nicht gesprochen werden, sondern nur von der Beschaffenheit der reziproken Kreuzungsprodukte.

Wir wollen nun zunächst einmal die verschiedenen phaenotypischen Möglichkeiten der Beschaffenheit solcher reziproken Kreuzungsprodukte kurz betrachten.

B. Der Phaenotypus reziprok verschiedener Bastarde

I. Zunächst ist es denkbar, dass die reziproken Verbindungen durchaus je dem einen oder dem anderen Elternteil gleichen. Die bekanntesten Beispiele hierfür bilden die sogenannten falschen Bastarde. Es seien hier genannt: Millardets *Fragaria-* und *Vitis*-Bastarde, Solms-Laubachs *Fuchsia*-Bastarde, Lidfors' *Rubus*-Kreuzungen und die Angaben von Haase-Besell für *Digitalis*.

In all diesen Fällen handelt es sich teils um vollkommene Patromorphie, teils um vollkommene Matromorphie. Dabei ist allerdings zu beachten, dass aus einer Kreuzung nicht immer lauter patro- oder matromorphe Formen hervorgehen, sondern neben den goneomorphen auch mehr oder weniger intermediäre; auch liessen sich nicht immer beide reziproke Verbindungen erzielen.

II. Die reziproken Verbindungen führen mit Hinblick auf einzelne Merkmale zu verschiedenen Erfolgen; so z. B. bezüglich der Ausbildung des Geschlechtes (*Bryonia*, *Lymantria*).

III. Die Patroklinie oder Matroklinie ist nicht vollkommen, sondern nur angenähert. Hier seien als Beispiele die *Digitalis*-Bastarde von Jones (Matroklinie) und die mannigfaltigen Oenotheren-Bastarde (z. B. zwischen *O. muricata* und *O. biennis*) (Patroklinie) genannt.

Es wird nun aber unsere Aufgabe sein, den Ursachen dieser Verschiedenheiten nachzugehen und sie zurückzuführen.

I. HETEROZYGOTIE UND HETEROGAMETIE

1. Vorgetäuschte Verschiedenheit

Es ist zweifellos, dass in nicht seltenen Fällen reziproke Verschiedenheit durch Heterozygotie der Ausgangsmaterialien vorgetäuscht wird, dadurch, dass die erblich komplizierten Materialien nicht genügend geklärt waren, die Aufspaltung unübersichtlich wurde und somit reziproke Verschiedenheiten irrtüm-

lich angenommen wurden. So wurden die reziproken Verbindungen von *Petunia nyctaginiflora* und *P. violacea* lange Zeit als verschieden angesprochen, von Correns aber schon 1901 (S. 190) als reziprok gleich festgestellt. Das Entsprechende gilt sicher für viele andere Verbindungen (*Ruellia* ?).

Heterozygotie braucht aber die reziproke Verschiedenheit der Bastarde nicht nur vorzutäuschen; sie führt auch in vielen besonders gelagerten Fällen zu echter reziproker Verschiedenheit. Das ist in allen den Fällen so, in denen es sich um Heterogamie, oder, um mit Bateson ('26) zu sprechen, um Anisogenie handelt.

2. Heterogamie oder Anisogenie

a. Wir betrachten als ein einfaches Beispiel dieser Art *Oenothera. O. Lamarckiana* ist heterogametisch, aber isogam. Wenn zwei Formen solcher Veranlagung miteinander gekreuzt werden, so gibt es reziprok gleiche Bastarde. Wenn aber von den verschiedenen Gameten beiderlei Geschlechtes immer die eine Art, und zwar in jedem Geschlecht jeweils die andere Art von Sexualzellen zu Grunde geht, ehe sie zur sexuellen Verbindung kommt, wie es tatsächlich bei *O. muricata* und *O. biennis* der Fall ist, so gibt es die bekannten reziprok verschiedenen Bastarde. De Vries ('11) nahm ja zuerst Vatergleichheit dieser Bastarde an; erst Davis ('14) betonte den Einfluss beider Eltern. Die ausgesprochene Patroklinie kommt durch die Dominanz des mit den väterlichen Gameten eingeführten Erbgutes nach beiden Richtungen zustande.

Andererseits kennen wir ja bei *Oenothera* auch durch Anisogenie hervorgerufene Matroklinie (vgl. z. B. bei Cobb die matroklinen Verbindungen von flat-revolute der *O. pratincola*).

b. Anisogenie und reziprok verschiedene Ergebnisse liegen, wie Correns für *Bryonia* zeigte, auch bei Verbindung monöcischer und diöcischer Pflanzen vor. Heterogametie im männlichen, Isogametie im weiblichen Geschlecht bei Dominanz der Diöcie führen hier zu reziproker Verschiedenheit. Weiter haben in Verbindung mit dem Geschlecht Bateson und Gairdner ('21) einen interessanten Fall am Flachs festgestellt. Gewöhnlich sind Flachsformen, Faser- wie Oelvarietäten, regelmässig zwittrig. Eine Zwergform aber, welche spontan auftrat, ist zwar gleicherweise zwittrig, doch gibt sie befruchtet von irgend einem anderen Faserflachs in F_2 eine rezessive Form, welche durch männliche Sterilität charakterisiert ist. Befruchtet durch Faserflachs gibt diese Form nur männlich sterile, während befruchtet vom Pollen des Zwerges in F_1 nur Zwittrige auftreten.

c. Allbekannt sind weiter die Untersuchungen von Frl. Saunders, welche zeigte, dass nur die Pollen von Levkojen in bestimmten Verbindungen die Eigenschaft des Gefülltseins übertragen, wodurch ebenfalls reziproke Verschiedenheiten bei Kreuzungen zustande kommen. Frl. Saunders ('11 und '16) wollte Anisogenie auch zur Erklärung des Gefülltseins bei *Petunia* heranziehen, doch hat Frl. v. Ubisch ('23) diesen Erklärungsversuch für diese Pflanze zurückgewiesen. Dagegen werden von Frl. Pellew ('15–'16) (vgl. dazu auch Bateson '26) für *Campanula carpatica* und von Bateson und Sutton ('19) für *Begonia Davisii* und *B. semperflorens* die Füllungserscheinungen ebenfalls mit Hilfe von Anisogenie erklärt.

d. Anisogenie liegt dann auch bei den bekannten Untersuchungen Blakeslees ('21) vor. Bei den *Datura*-Mutanten Globe und Poinsettia wird das Extrachromosom nur durch den Pollen übertragen. Der Erfolg der reziproken Kreuzungen ist dann also

	Poinsettia × Normal	Normal × Poinsettia
N	2864	1126
P	1304	0

Ganz entsprechend, wenn auch nicht so ausgeprägt liegen die Verhältnisse nach Overeem ('23) für *O. semigigas*, wo auch der Pollen hauptsächlich die Normalchromosomigen, uberträgt, wie auch die Eizellen dieselben übertragen.

Denkbar ist dann auch bei Pflanzen, dass crossing over bei der Microsporen oder Megasporenbildung häufiger ist. Bei Mais wurden diese Verhältnisse von Emerson ('21) und von Eyster ('22) studiert, doch wurden keine erheblichen Differenzen festgestellt, ebenso von Altenburg bei *Primula sinensis*. Dagegen fanden Gowen ('19) und Gregory und Bateson höhere crossing-over Prozente in den Eiern, was zu einer reziprok verschieden zusammengesetzten Nachkommenschaft führt.

e. Das Problem in allen diesen Fällen liegt natürlich in der Frage: *Wie kommt diese Heterogamie oder Anisogenie entwickelungsphysiologisch zustande?* Wir können natürlich das Vorhandensein von Lethalfaktoren, grössere Empfindlichkeit der männlichen Gameten, entwickelungsgeschichtliche Sterilität, Gonenkonkurrenz, etc. annehmen. Die ganze Problematik aber wird besonders schön durch Batesons ('26) Rogue-Kreuzungen bei Erbsen aufgeworfen. Bateson findet ja hier bekanntlich, dass die Rogue-Eigenschaften während der Entwickelung der einzelnen Pflanzen immer mehr hervortreten. Das wurde besonders bei nicht ganz typischen rogue-intermediates der Varietät Gradus festgestellt, aber auch bei echter rogue. Dabei stellte sich nun durch reziproke Kreuzungen heraus, dass die Zunahme der rogue Eigenschaft auf männlicher Seite viel schneller vor sich geht, als auf weiblicher Seite und Bateson nimmt an, dass bei den somatischen Teilungen während der Ontogenese die unterschiedliche Ausbildung vor sich geht.

Verlassen wir aber nun die Fälle, in denen es sich um Heterozygotie oder Heterogametie, die zu reziprok verschiedenen Kreuzungsresultaten in irgend welcher Form führen, handelt und wenden uns zu homozygotischen Formen, welche bei reziproker Verbindung verschiedene Bastarde liefern.

II. HOMOZYGOTIE

In den Fällen, wo reziproke Verschiedenheit bei Verbindung homozygoter Ausgangsmaterialien zustande kommt, kann die Verschiedenheit zunächst rein mütterlich bedingt sein.

1. Rein mütterliche Bedingtheit der reziproken Verschiedenheiten

a. *Einfluss von Frucht und Samen.* Wir wollen nicht lange bei einem mehr mechanischen Einfluss von Frucht-und Samenschale, also einem rein mütterlichen Einfluss verweilen. Derartige Einflüsse sind ja für Mais (Correns '01),

für Erbsen und Bohnen (Tschermak '01) festgestellt worden. Neuerdings werden solche Einflüsse auch von Hill ('25) für reziproke Verschiedenheiten von Kotyledonarmerkmalen bei Kreuzungen von *Digitalis*-Arten angenommen.

b. *Eiplasma.* Dass weiter dem Eiplasma seit Boveri ('92) und Driesch ('98) vielfach ein bestimmender Einfluss auf embryonale und larvale Stadien zugesprochen wird, welcher reziproke Verschiedenheit bedingt, ist bekannt und soll hier, da die Untersuchungen sich ja auf tierischem Gebiet abspielen, ebenfalls nicht weiter erörtert werden. Auch an den Einfluss des Eiplasmas auf die Furchung und damit zusammenhängende Eigenschaften, wie die Windungen der Gastropoeden (Sturtevant '23) soll hier nur erinnert werden.

c. *Bastardendosperme.* An die mütterliche Vererbung lassen sich, wenn auch in anderem Sinne, dann die reziproken Verschiedenheiten anschliessen, welche sich in den Bastardendospermen vom Mais haben feststellen lassen. Es wurde zuerst von Correns (S. 95) in überzeugender Weise gezeigt, dass die weibliche Erbmasse des Endosperms der männlichen stets durch die beiden Embryosackkerne überlegen sei, sodass, wenn nicht eine sehr starke Dominanz des von männlicher Seite eingeführten Merkmales in Frage kommt, ausgeprägte Matroklinie des Bastardendosperms zu beobachten ist. Es ist bekannt genug, wie in ihren eingehenden Versuchen weiterhin bis in die neuste Zeit East, Hayes, und Jones gezeigt haben, wie die verschiedenen Bestandteile des Endosperms durch diese Kernverhältnisse beeinflusst werden. Wir können auf die Einzelheiten hier nicht eingehen. Diese reziproken Verschiedenheiten machen sich ja aber im Endosperm auch, abgesehen vom Mais, noch bei Weizen (Correns S. 96, Sax '22) geltend.

d. *Apogamie.* Reziprok verschieden, und zwar rein metroklin müssen dann natürlich auch die Kreuzungsergebnisse bei apogamen Formen ausfallen, ganz gleich ob diploide Eier sich ohne allen Befruchtungsreiz weiterentwickeln (*Antennaria*, Juel; *Alchemilla*, Strasburger), oder erst angeregt durch den Pollenschlauch (*Thalictrum*, Overton '04; *Atamasco*, Pace '13; *Zygopetalum* u. *Odontoglossum*, Süssenguth '23).

Besonders auffallend aber wird diese matrokline Entwicklung, wenn, wie bei *Hieracium* (Rosenberg '07) neben haploiden, befruchtungsbedürftigen Eiern apogam sich entwickelnde auftreten und somit neben intermediären rein matrokline "Kreuzungsprodukte" entstehen. Nach dieser Richtung besonders interessant sind ja auch die Versuche Digbys und Pellews mit ihren Kreuzungen zwischen *Primula floribunda verticillata* bezw. *Kewensis.* Diese Autoren fanden, dass bei Kreuzung dieser Arten untereinander mit geringen Ausnahmen immer nur matromorphe Bastarde entstanden, welche vom Vater keine Merkmale erhielten. Sie haben aber den Schluss, dass es sich in diesem Falle um apogame Entwicklung der Eizellen, welche durch Bestäubung mit dem artfremden Pollen angeregt wird, dadurch zu stützen vermocht, dass schon bei sorgfältigster Kastrierung die Arten auch ohne Bestäubung apogam vereinzelt Nachkommen hervorbrachten.

Die auf tierischem Gebiet seit Loeb ('07) ausgeführten Untersuchungen über Aktivierung von Parthenogenese in Verbindung mit Kreuzung werden sich

vielleicht auch auf pflanzlichem Gebiet und vielleicht gerade für die Frage der reziprok verschiedenen Bastarde auswerten lassen. Vielleicht gehören hierher schon die von Blakeslee beschriebenen, auf Kältewirkung zurückgeführten haploiden Daturen, Manns haploide Nicotianen, etc. Auch ist natürlich daran zu denken, dass Fälle des Ausschlusses männlicher Chromosomen, wie sie Baltzer für Echinidebastarde feststellte, ebenfalls hier eine Rolle spielen könnten.

2. Von männlicher Seite bedingte reziproke Verschiedenheit

a. *Falsche Bastarde.* Nicht so einfach liegen die Verhältnisse bei den *patromorphen* Bastarden, wie sie ja unter den sogenannten falschen Bastarden verschiedentlich beschrieben wurden. Schon Giard wollte bekanntlich die faux hybrides Millardets durch Merogonie erklären und diese Versuche der Merogonieerklärung spielten ja auch sonst auf pflanzlichem Gebiete keine ganz untergeordnete Rolle, wenn sie auch auf tierischem Gebiete seit Boveri viel erfolgreicher verwandt werden konnte. Denn eine wirklich einwandfreie Merogonieerklärung patromorpher Bastarde auf pflanzlichem Gebiete liegt m.W. ja nicht vor. Hat ja auch Strasburger ('09, Histol. Beitr. 7) die rein väterlich erscheinende Verbindung *Fragaria virginiana*×*elatior* histologisch untersucht und findet echte Befruchtung, sodass er schliesst "dass in bestimmten Fällen die erblichen Merkmale des einen der beiden Kerne, die im Befruchtungsakt zur Vereinigung kommen, ganz über die des anderen dominieren können." Und auch sonst hat sich bei den höheren Pflanzen die Merogonieerklärung für patrokline Bastarde niemals stichhaltig erwiesen.

b. *Chloroplastenvererbung.* Auf die Fülle und Mannigfaltigkeit aller der Fälle von Uebertragung von Chloroplastencharakteren hier einzugehen, ist nicht der Ort. Sehen wir ganz ab von infektioser Panaschen-und Chimärennatur, so wissen wir doch durch die Untersuchungen der allerverschiedensten Autoren, dass hier einfache mendelistische Erbübertragung für einfache Färbungsunterschiede, wie für Scheckung (Correns '20; Bateson, *Tropaeolum*, '20) neben komplizierteren Mendelfällen (Miles, Emerson, Demerec, Nilsson-Ehle, Trow, Lindstrom, etc.) in Frage kommen kann.

Daneben ist ja aber auch genugsam bekannt, dass scharf ausgesprochene reziproke Verschiedenheiten der Vererbung auftreten können, die dann immer matroklin sind. Der erste Fall dieser Art ist wohl die *Mirabilis albomaculata* von Correns. Hier führt die Kreuzung von weissbunt ♀×normal grün ♂ zu weissbunt, umgekehrt zu normal grün. Entsprechende Fälle sind Baurs *Antirrhinum*, Gregorys *Primula sinensis*, Shulls *Melandrium*, Andersons gestreifte Maisvarietäten.

In Verbindung mit der Kreuzung zwischen verschiedenen Arten und zur Klärung der Kreuzungsresultate kommen diese Verhältnisse dann bei den Oenotheren, z.B. durch Renners ('24) Versuche bei der Kreuzung von *O. Hookeri* ×*Lamarckiana* in Frage. Er hat eine scharfe Trennung von Kern und Plasma für seine Hookeri Kreuzungen durch Generationen erwiesen.

Die Meinungen, ob dieses Nichtergrünen auf plasmatische Einflüsse zurückzuführen ist oder ob der Grund in den Chloroplasten selbst zu suchen ist, sind

etwas geteilt. Correns neigt dazu, das Plasma verantwortlich zu machen, Gregory ('16, '17) verlegt den Sitz in die Chloroplasten, Baur ('09) und Winge ('19) ziehen beide Möglichkeiten in Betracht. Renner nimmt zur Erklärung seines Falles an, dass bei Hookeri×Hookeri bezw. velans×Hookeri die Chromatophoren in dem betreffenden Plasma nicht zu ergrünen im Stande sind. Der Fall mütterlicher Vererbung, welchen Terao ('18) für die Chloroplastenfärbung von Soja angibt, wird von Woodworth ('21) mendelistisch gedeutet.

Es gibt ja aber nun gerade auf diesem Gebiete die allergrösste Mannigfaltigkeit und nicht selten treten Schecken auf. Diese Schecken werden oft durch Uebergang von albinotischen Chloroplasten oder krankem Plasma aus dem Pollenschlauch in die Eizelle zustandekommend erklärt, wodurch also dann die reziproke Verschiedenheit verwischt wird (Baur '09, Ikeno '16, Ishikawa '18, Winge '17, Renner '22). Von anderen Seiten wurden diese Schecken aber auch durch ontogenetisch-entwickelungsgeschichtliches Geschehen erklärt (Noack).

3. Plasmaeinwirkung bei allgemeinen Verschiedenheiten reziproker Bastarde

Dass reziproke Bastarde in sehr verschiedenen Merkmalen voneinander abweichen können, wurde schon eingangs auf Grund der älteren Untersuchungen (Kölreuter, Gärtner, etc.) dargelegt. Dass solche reziproke Verschiedenheiten vielleicht auf plasmatischer Basis erklärt werden könnten, da durch die Eizelle neben dem Kerne grössere Plasmamengen, durch den Pollen nur der Kern geliefert wird, wurde schon von Strasburger ('84) dargelegt. In neuerer Zeit ist diese Anschauung in Verbindung mit experimentellen Untersuchungen in erster Linie von Jones ('12) bei seinen *Digitalis*-Bastarden vertreten worden.

Andererseits ist mir auf pflanzlichem Gebiete noch kein Fall bekannt geworden, bei dem diese reziproken Verschiedenheiten durch plasmatische Erklärungen wirklich erwiesen worden wären. Es erschien aber doch besonders wichtig, dieser Frage einmal wirklich näherzutreten, da ja die reziproken Bastarde, wie besonders Winkler ('23) ausführte, das wertvollste Mittel sein dürften, die Beteiligung des Plasmas an der Vererbung weiter zu klären.

Ich hatte nun zunächst reziproke Kreuzungen in der Gattung *Epilobium* angestellt, um die Beziehungen im Vererbungsgang dieser Gattung zu der naheverwandten *Oenothera* zu klären. Es lag nun aber nahe, diese Kreuzungen auch unter den hier erörterten Gesichtspunkten zu studieren.

Reziproke Kreuzungen in der Gattung Epilobium

Es war früher die Meinung unter einer Anzahl von Systematikern verbreitet (Schultz bipontinus, Léveillé) dass die Bastarde zwischen verschiedenen *Epilobium*-Arten immer reziprok verschieden seien, ja, dass man einem in der freien Natur gefundenen Bastard sogar ansehen könne, welchen Elternteil, Vater oder Mutter, er darstelle. Durch eingehende experimentelle Untersuchung liess sichaber in neuester Zeit die merkwürdige Tatsache feststellen, dass die Bastarde zwischen den meisten *Epilobium*-Arten reziprok gleich sind; nur immer dann—mit seltenen anderen Ausnahmen—wenn *E. parviflorum* oder *E. hirsutum* in die Kreuzung eingehen, hat sich reziproke Verschiedenheit erweisen lassen. Diese

reziproke Verschiedenheiten sind teilweis sehr stark ausgeprägt. Sie bestehen im folgenden:

Immer dann, wenn *E. parviflorum* oder *E. hirsutum* Mutter ist, ganz gleichgültig, soweit unsere bisherigen Untersuchungen reichen, welche andere Art als Vater in Frage kommt, treten im Bastard Hemmungen auf, welche der reziproken Verbindung fehlen. Diese Hemmungen machen sich teils in der vegetativen, teils in der reproduktiven Sphäre geltend. Es handelt sich um Höhenverschiedenheiten, Blattgrössen, Blütengrössen, Pollen- und Samensterilität (Fig. 1).

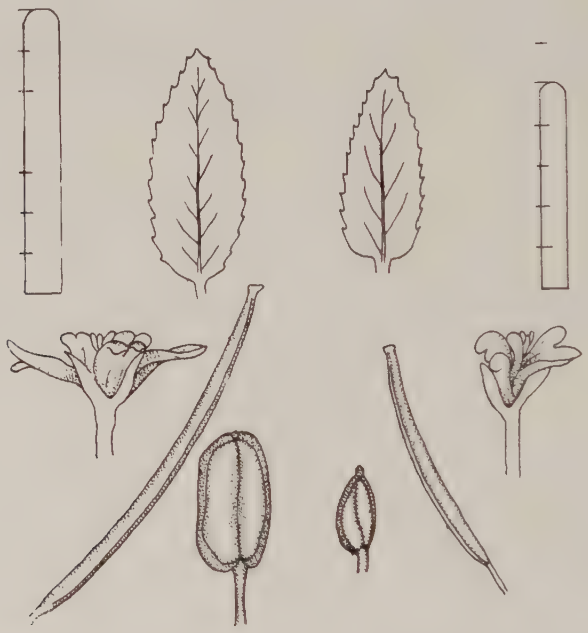

Fig. 1. *E. hirsutum* oder *E. parviflorum* ♂ × einer anderen *Epilobium*-art. *E. hirsutum* oder *E. parviflorum* ♀ × einer anderen *Epilobium*-art.

Ausserdem tritt eine Verschiebung einzelner Merkmale, wie Blattgestalt nach der mütterlichen Seite in Erscheinung.

Auf dem Boden der Plasmatheorie zur Erklärung der Verschiedenheiten reziproker Bastarde wäre also anzunehmen, dass der Kernanteil der übrigen *Epilobium*-arten im Plasma von hirsutum und parviflorum nicht günstige Lebensbedingungen fände (Fig. 2). Darauf wären alle die Hemmungserscheinungen zurückzuführen, darauf könnte man auch zurückführen, dass die väterlichen Eigenschaften im parviflorum bezw. hirsutum, bei dem eine *einfach* plasmatische Erklärung ja überaus nahegelegen hätte, dieselbe versagt und eine chromosomale, faktorielle Basis zur Erklärung notwendig macht (vgl. Fig. 4).

Nun konnte aber Schwemmle noch weiter feststellen, dass die Steril-fertil-Klassen in ganzen Pflanzen wie Samen nicht gleich gross waren, vielmehr war die Sterilität bei gleicher Kernkombination im Plasma 2 stets höher als im Plasma

1, das zeigte sich noch besonders bei einer Kombination mit einem dritten roseum (3). (Fig. 5A.)

Auf Grund dieser Feststellung kommt man nun zu der Annahme, dass der Kern nicht *allein* die Sterilität bestimmt, sondern dem Plasma eine verschiedene Rolle zukommt.

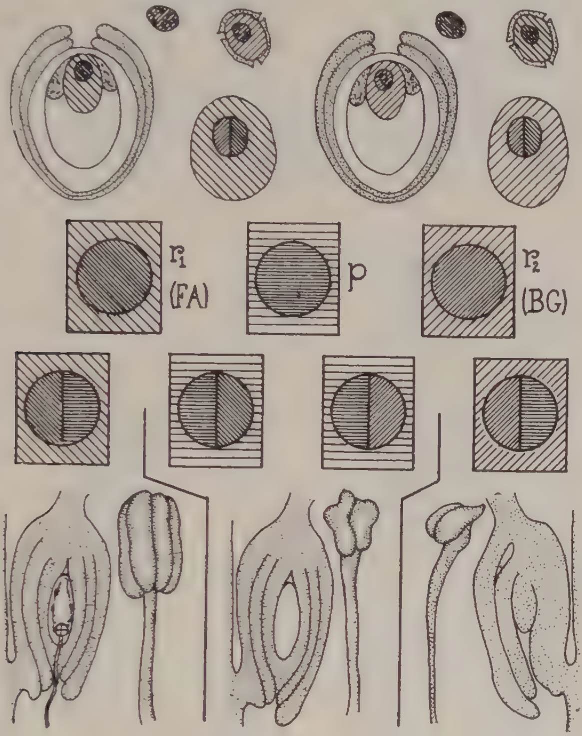

Fig. 2. Kreuzung von *E. parviflorum* (p) mit *E. roseum* 1 (r_1) und *E. roseum* 2 (r_2) und die Sterilitätsverhältnisse der F_1.

Wie aber ist diese verschiedende Wirkung zu denken? Schwemmle nimmt an, ganz in Uebereinstimmung mit einer älteren Angabe von Correns aus dem Jahre 1903, dass bei reziproken Verschiedenheiten von Bastarden, das Plasma vom Kernanteil beeinflusst sei und somit die Plasmabeschaffenheit gleichsam über den Kern verändert würde. Schwemmle hat auch in seinen Untersuchungen einen Anhalt für diese Annahme. Stellt er nämlich dieselbe Kernkombination roseum 1 $\times$parviflorum her, so ist sie dann steriler, wenn sie über eine heterozygote Verb. mit roseum 2 erstellt würde, als direkt mit der Homozygote roseum 1. Schwemmle nimmt an, dass der Chromosomanteil von roseum 2 das Plasma von roseum 1 schon derart verändert hat, dass eine Hemmung ausgeübt wird (Fig. 5B).

Bemerkenswert ist auch, und spricht ganz im Sinne einer stattfindenden Einwirkung der Kernanteile auf das Plasma, dass junge Pflanzen mit parviflorum Plasma steriler sind und auch in den anderen Hinsichten stärker gehemmt als

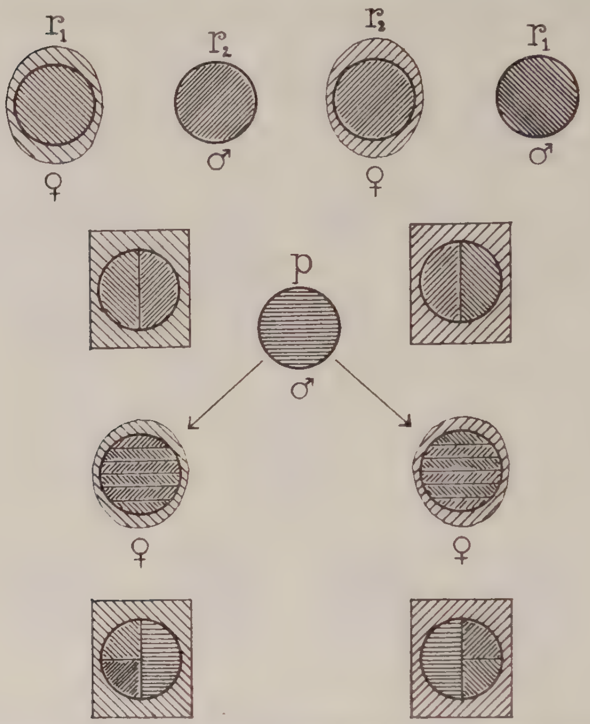

Fig. 3. Ergebnisse der Einkreuzung von *E. parviflorum* in die reziproken Kreunzungen von *E. roseum* (1) und *E. roseum* (2), dargestellt für Zutreffen der Plasmatheorie.

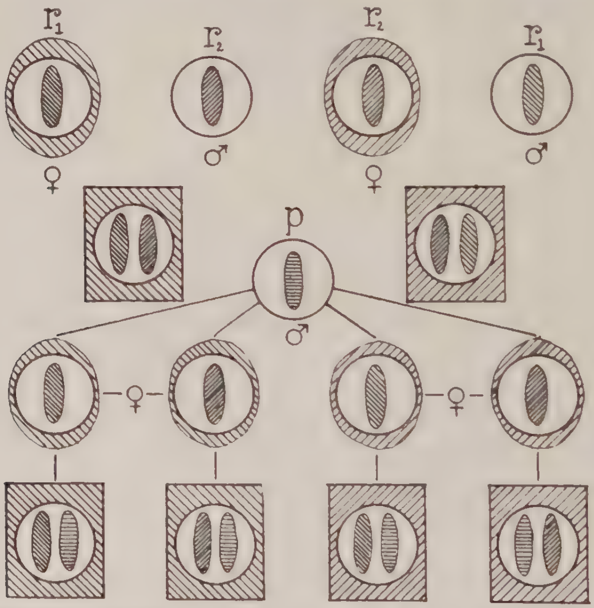

Fig. 4. Tatsächliche Ergebnisse der Einkreuzung von *E. parviflorum* in die reziproken Kreuzungen von *E. roseum* (1) und *E. roseum* (2) schematisch dargestellt.

ältere. So werden auch in späteren Lebensjahren die Blüten grösser, ja es treten erst ganz sterile Pflanzen vereinzelten Samen, bei teilweise fertiler erhöhter Fertilität, auf.

Wie aber erklären nun diese Versuche die reziproke Verschiedenheit der parviflorum und hirsutum-Bastarde? Auch hier kommt nach Schwemmle die Hemmung durch faktorielle Grundlage zustande. Die Hemmungsfaktoren sind aber verschieden stark. Parviflorum hat viel stärkere Hemmungsfaktoren als roseum;

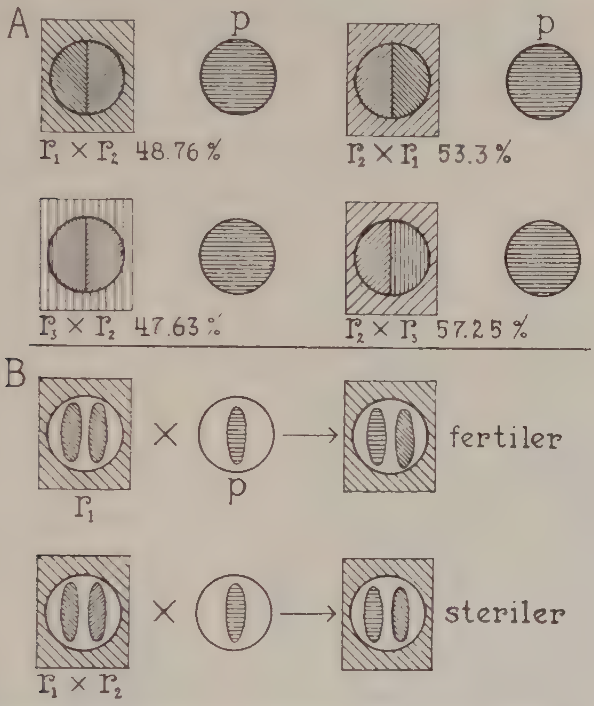

Fig. 5. Verschiebung der Sterilität der reziproken Bastarde von *roseum* (1) and (2) bezw. (3), gekreuzt mit *parviflorum* in schematischer Darstellung. A, Sterilitätsprozente der verschiedenen Verbindungen. B, die Heterozygote r_1 *parviflorum* auf dem Weg über homozygotische, bezw. heterozygotische Verbindung (mit r_2) gewonnen.

dass sie auch bei roseum schon verschieden stark sein können, zeigen ja unsere Versuche. Wenn parviflorum weiblich mit roseum männlich verbunden, so haben wir ein Plasma, auf welches der Kern von parviflorum mit starken Hemmungen schon lange eingewirkt hat; das Plasma wird hemmende Substanzen enthalten; der roseum-kernanteil wird stark gehemmt sein. Im umgekehrten Falle kommt roseum-plasma in Frage, in dem der roseum-Kern mit viel geringer wirksamen Hemmungen gelebt hat; der parviflorum-Kern wird also nicht stark gehemmt und die Verbindung ist wenig gehemmt.

Schwemmle kommt also hier zu ganz ähnlichen quantitativen Vorstellungen für die reziproke Verschiedenheit wie Goldschmidt für seine Intersexe.

Diese Annahme kann man nun wohl machen (Renner und Kupper '21), aber es war nötig, sie auch zu erweisen, wie ich schon vor einer Reihe von Jahren ausein-

andersetzte. Dieser Aufgabe unterzog sich nun Schwemmle in meinem Institut auf Grund einer eigenen, von ihm ausgearbeiteten Fragestellung. Er ging dabei auf einige meiner roseum-parviflorum Kreuzungen zurück. Diese verhielten sich nämlich in der Regel durchaus nach dem eben besprochenen Schema: Sterilität mit parviflorum als Mutter, Fertilität im umgekehrten Falle. Bei Verwendung eines roseum-Biotypus— es gibt deren zweifellos eine ganze Reihe—zeigte es sich nun, dass auch eine Verbindung von roseum weiblich mit parviflorum männlich auftreten kann, in der Sterilität zu beobachten ist. Es ergab sich dann also das Folgende:

Roseum 1 Mutter × parviflorum Vater ist steril. Es wäre also auch hier auf dem Boden der Plasmatheorie anzunehmen, dass auch der Kernanteil von parviflorum im Plasma von roseum 2 keine günstige Entfaltungsmöglichkeit findet (Fig. 2).

Schwemmle stellte nun die folgende Ueberlegung an. Es wäre diese Plasmatheorie auf ihre Richtigkeit zu prüfen, wenn mit parviflorum als männlichem

FIG. 6. *a, Epilobium roseum × E. hirsutum; b, E. hirsutum × E. roseum.*

Elter in die reziproken Verbindungen der beiden roseum-Formen eingekreuzt wurde. Dann befinden sich die beiden gleichen roseum-Kernanteile gewiss in verschiedenem Plasma; liegt die Sterilität der Verbindung roseum 2 × parviflorum wirklich daran, dass der parviflorum-Kernanteil sich im Plasma von roseum 2 nicht gut entfalten kann, so mussten also die Verbindungen mit parviflorum dann stets steril sein, wenn das Plasma von roseum 2 vorhanden ist; wenn Plasma von roseum 1 vorliegt, aber müssten fertile Verbindungen beobachtet

werden. Immer ist dabei zu berücksichtigen, dass die roseum-Verbindungen selbst stets voll fertil sind und parviflorum gleichsam als Reagens auf die Beschaffenheit des roseum-Plasma zur Verwendung kommt (vgl. Fig. 3).

Es zeigt sich nun aber, dass die Erwartung nicht eintrifft. Wir sehen bei der Verbindung von parviflorum männlich mit den beiden roseum-Heterozygoten-Formen weiblich stets eine Spaltung in ungefähr zur Hälfte fertiler und zur anderen Hälfte steriler Pflanzen auftreten.

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MULTIPLE ALLELOMORPHS VERSUS MULTIPLE FACTORS[1]

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In the twenty-five years of its existence, genetics has gained an important position in the society of sciences. Other members of this society are admitting more and more its equivalence, and not only those of nearest relationship, that is, from the circle of biological, medical, and agricultural sciences, but also sociology and pure jurisprudence, even philology, begin to take notice of its results. Advices from geneticists have been taken in some disputable law-suits. Very recently (1926) a philologist in Holland, Professor van Ginneken, has tried to adapt the study of phonetics to the various theses of genetics. Many phenomena discovered by geneticists, such as modifications, mutations, and Mendelian segregations have been distinguished by him in the historical development of a number of languages, as consequences of the same processes in the structure of human organs of pronunciation.

Such a prominent part as genetics is now playing among its fellows charges its tutors with a great responsibility; we have therefore the duty to make its character reliable, and above all, to be careful in the verdicts that we are publishing in the name of genetics. It is my personal feeling that genetics at present is working somewhat superficially; we must try to deepen its foundations, and from time to time we must undertake a thorough revision of its theses.

In the course of the last fifteen years, we have learned to understand that the simple numerical proportions pointed out by Mendel in his experiments with peas do not occur generally; they may even be considered to form exceptional cases. We have followed the lines indicated by his genius, and when our numerical proportions were more complicated, we have nearly always succeeded in building up an explanation of this greater intricacy. At the present time we dispose of a lot of hypotheses which can clear up these deviations from Mendel's original proportions. We are accustomed to work with cryptomery or inactive factors, with multiple factors, with selective fertilization, with lethal factors in gametes and in zygotes, with irregularities of chromosomal distributions, as non-disjunction, deficiency, etc. In nearly all experiments we can find among these various assumptions one which agrees rather well with the observed ratios, and when this is the case, we are inclined too much to accept this assumption as a true and undoubted explanation.

Among these hypotheses, that of the multiple factors (polymery in the European literature) takes a prominent position. According to this assumption

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all segregations in the F_2 generations may follow the theoretical proportions of 15:1 for duplicate factors; of 63:1 for triplicate; 255:1 for quadruplicate; and 1023:1 for quintuplicate factors. In back-crosses with the recessive these proportions are, for the same number of factors, 3:1, 7:1, 15:1, and 31:1. In genetical literature these proportions are considered to be caused by two or more independently segregating factors, localized, therefore, in different chromosomes. In my opinion, however, this explanation is rather doubtful.

I will submit for your consideration the results of a research into the inheritance of an aberrant form of *Lamium album*, the white dead-nettle. Last year I published some of these results in the Dutch periodical "Genetica," but since they have been extended somewhat.

The aberrant form with peloric top-flowers in *Lamium album* is shown by selfing to be entirely constant. Three beds contained 268 plants, all pelorics. Crosses with normal plants produced a number of F_1 generations, consisting of 1106 plants, which showed the normal appearance to be the dominant one. Five F_1 plants were used for breeding offspring; they were partly selfed, partly back-crossed with the recessive peloric form in both directions. The offspring of these F_1 plants were grown separately, as well as the F_2 generations, the $F_1 \times R$, and the $R \times F_1$ back-crosses. The numerical proportions obtained are compiled in the following table:

From these numbers it is apparent that one of the five F_1 plants showed in its F_2 generation a clear segregation of 15:1, while both its back-crosses split up as 3:1; a second plant segregated in F_2 about 63:1, in the back-crosses 7:1; a third gave an F_2 generation of 318 normals to 1 recessive, and in the $F_1 \times R$ segregations it showed a proportion of 13.15:1; the fourth in F_2 269:1 and in the back-crosses 13.43:1; while the fifth yielded an F_2 generation of 275, all normal individuals and back-crosses, segregating accordingly 21.30:1.

From an orthodox Mendelian viewpoint one should like to explain these proportions by assuming a number of multiple factors, each causing the normal form of inflorescence. The first mentioned plant would then possess two of these factors (F_2 15:1 and F_1R 3:1); the second would be heterozygous in three factors (F_2 63:1; F_1R 7:1); the third and fourth must be supposed to be heterozygous in four factors (F_2 255:1 and F_1R 15:1) and the fifth possibly in five factors (F_2 1023:1 and F_1R 31:1). As a second hypothesis we are obliged to assume that the normal parent used possessed the whole of these five factors, three of them being heterozygous. But supposing this state of things, the first two plants only fit well the expectations. The third and fourth have yielded a marked deficit of dominant normal plants (13.15:1 and 13.43:1 instead of 15:1 in the back-crosses), while the fifth produced a still more deficient number (21.30:1 instead of 31:1). This deviation from the theoretical explanation compels the orthodox Mendelian geneticist to search for an explanation. Lethal factors cannot be supposed to be present, for the percentage of failing pollinations, or that of the non-germinating grains, is not greater than that in other regularly segregating families; selective fertilization is absent, for the phenomenon is observed as well in crosses in which the recessive peloric form func-

TABLE 1. LAMIUM ALBUM NORMAL×PELORIC. 1231 IS PELORIC FORM; 1238 AND 1240 ARE F_1-FAMILIES

Parent	Year	Pedigree Number	Number of Flowers	Number of Seeds	Number of Plants	Normals	Pelorics	Numerical Proportion
1922.1238.1 selfed	1923	568	43	159	151	151	0	
	1924	630	48	179	168	167	1	
				Sum	319	318	1	
1922.1238.1×1231	1923	569	29	109	101	93	8	11.62:1
	1924	631	38	140	129	120	9	13.33:1
	1925	391	91	346	330	307	23	13.35:1
				Sum	560	520	40	
1922.1231×1238.1	1923	570	17	61	59	53	6	8.83:1
	1924	632	26	99	93	89	4	22.25:1
	1925	392	67	251	236	219	17	12.88:1
				Sum	388	361	27	
		Total of backcrosses			948	881	67	13.15:1
1922.1238.2 selfed	1923	571	50	188	177	164	13	
	1924	633	37	139	128	122	6	
				Sum	305	286	19	
1922.1238.2×1231	1923	572	24	87	81	59	22	2.68:1
	1924	634	31	116	112	88	24	3.66:1
				Sum	193	147	46	
1922.1231×1238.2	1923	573	36	135	127	95	32	2.97:1
	1924	635	19	68	61	43	18	2.39:1
				Sum	188	136	50	
		Total of backcrosses			381	285	96	2.97:1
1922.1238.3 selfed	1923	574	41	147	134	134	0	
	1924	636	42	156	141	141	0	
				Sum	275	275	0	
1922.1238.3×1231	1923	575	29	109	96	91	5	18.20:1
	1924	637	27	93	81	77	4	19.25:1
	1925	393	62	309	293	279	14	19.93:1
				Sum	470	447	23	
1922.1231×1238.3	1923	576	30	102	97	94	3	31.33:1
	1924	638	28	100	92	87	5	17.40:1
	1925	394	121	457	435	416	18	20.53:1
				Sum	624	597	26	
		Total of backcrosses			1093	1044	49	21.30:1
1922.1240.1 selfed	1923	577	37	134	125	123	2	
	1924	639	46	171	162	160	2	
				Sum	287	283	4	

TABLE 1—*Continued*

1922.1240.1×1231	1923 1924	578 640	28 34	101 125	95 120	82 107	13 13	6.31:1 8.23:1
				Sum	215	189	26	
1922.1231×1240.1	1923 1924	579 641	37 32	130 115	121 107	104 97	17 10	6.12:1 9.70:1
				Sum	228	201	27	
		Total of backcrosses			443	390	53	7.36:1
1922.1240.2 selfed	1923 1924	580 642	27 50	103 181	98 172	98 171	0 1	
				Sum	270	269	1	
1922.1240.2×1231	1923 1924 1925	581 643 395	34 31 98	129 111 364	122 102 338	116 93 314	6 9 24	19.33:1 10.33:1 13.08:1
				Sum	562	523	39	
1922.1231×1240.2	1923 1924 1925	582 644 396	38 16 67	135 51 259	120 44 241	110 42 225	10 2 16	11. 0:1 21. 0:1 14.06:1
				Sum	405	377	28	
		Total of backcrosses			967	900	67	13.43:1

tioned as the male parent; the only possible explanation then is the supposition that two of the five multiple factors are linked.

If we accept this assumption, it is necessary to calculate the influence exercised by such a linkage on the numerical proportions of multiple factors; a table may show the theoretical numbers expected in the F_2 generations and in the F_1R back-crosses on the assumption that 2, 3, 4 or 5 multiple factors are present, two of which are linked in various intensities (from independent, 1AB:1Ab, to complete linkage ∞ AB:1Ab). From this table it is clear that a linkage of an intensity 3AB:1Ab (or 25 per cent crossovers) must be assumed to explain the proportion of 21:1 in the back-crosses between a quintuple heterozygous plant with the recessive one; while a linkage of about 1.5:1 (or 40 per cent crossovers) is necessary to cause the proportion of 13:1 in the quadruple back-cross.

Along these lines a purely orthodox Mendelian interpretation of the observed deviations really could be established; and so the first case, as far as I know, of a distinct linkage between two out of a series of multiple factors would be discovered.

Nevertheless, I do not take the liberty of proposing to you this solution of the problem as the most acceptable one. If we agree with the hypothesis of a supposed number of multiple factors, some of which are linked to a supposed degree, we can explain immediately every numerical proportion observed already or to be observed in future work. Assuming the existence of duplicate factors, we get in F_2 all gradations between 15:1 and 3:1, according to the link-

age-intensity, while in the back-crosses the gradations vary between 3:1 and 1:1. In this way every numerical proportion in F_2 and in F_1R can be explained by the assumptions of a certain number of multiple factors and a certain intensity of linkage. That will lead *ad absurdum*, and this must be a serious warning against a too ready assumption of this stage of things.

Reviewing in the genetical literature the data which are interpreted as processes of independent multiple factors, we must state that a nearly exact agreement of the observed proportions with the theoretical expectations is a rare exception. In 1914 Kajanus submitted the literature of that time to a searching criticism; I should not like to follow him in all his arguments, but I wish to take

TABLE 2. NUMERICAL PROPORTIONS IN F_2-GENERATIONS OF HETEROZYGOTES WITH 2, 3, 4, 5 DOMINANT MULTIPLE FACTORS, TWO OF WHICH (A AND B) ARE LINKED

SELF-POLLINATION

	2 Fact. AaBb	3 Fact. AaBbCc	4 Fact. AaBbCcDd	5 Fact. AaBbCcDdEe
1AB:1Ab	15.00:1	63.00:1	255.00:1	1023.00:1
2AB:1Ab	8.00:1	35.00:1	143.00:1	575.00:1
3AB:1Ab	6.11:1	27.44:1	112.77:1	454.11:1
4AB:1Ab	5.25:1	24.00:1	99.00:1	399.00:1
5AB:1Ab	4.76:1	22.04:1	91.16:1	367.64:1
6AB:1Ab	4.44:1	20.77:1	86.11:1	347.44:1
7AB:1Ab	4.22:1	19.90:1	82.59:1	333.37:1
8AB:1Ab	4.06:1	19.25:1	80.00:1	323.00:1
9AB:1Ab	3.93:1	18.75:1	78.01:1	315.05:1
10AB:1Ab	3.84:1	18.36:1	76.44:1	308.76:1
11AB:1Ab	3.76:1	18.04:1	75.16:1	303.64:1
12AB:1Ab	3.70:1	17.77:1	74.11:1	299.44:1
∞AB:1Ab	3.00:1	15.00:1	63.00:1	255.00:1

BACKCROSSES WITH RECESSIVE

	2 Fact. AaBb	3 Fact. AaBbCc	4 Fact. AaBbCcDd	5 Fact. AaBbCcDd
1AB:1Ab	3.00:1	7.00:1	15.00:1	31.00:1
2AB:1Ab	2.00:1	5.00:1	11.00:1	23.00:1
3AB:1Ab	1.66:1	4.33:1	9.66:1	20.33:1
4AB:1Ab	1.50:1	4.00:1	9.00:1	19.00:1
5AB:1Ab	1.40:1	3.80:1	8.60:1	18.20:1
6AB:1Ab	1.33:1	3.66:1	8.33:1	17.66:1
7AB:1Ab	1.28:1	3.57:1	8.14:1	17.28:1
8AB:1Ab	1.25:1	3.50:1	8.00:1	17.00:1
9AB:1Ab	1.22:1	3.44:1	7.89:1	16.77:1
10AB:1Ab	1.20:1	3.40:1	7.80:1	16.60:1
11AB:1Ab	1.18:1	3.36:1	7.72:1	16.45:1
12AB:1Ab	1.17:1	3.33:1	7.66:1	16.33:1
∞AB:1Ab	1.00:1	3.00:1	7.00:1	15.00:1

into consideration the emphatical lesson he taught us. The exciting example of Nilsson-Ehle, who gave guidance in a disordered mass of highly divergent numerical proportions, has seduced many geneticists to press such proportions into the scheme 3:1, 15:1, 63:1, etc. It need not be emphasized here that thus the way to the most arbitrary dealings is opened. Taking as an example the

numbers in Ehle's own researches, those observed in F_2 and in F_3 generations of a cross between red and white wheats, we find that the F_2 generation segregated in the proportion of 14.7:1, and this was therefore considered as a dihybrid; the F_3 families showed a whole series of proportions as seen from the table below. From these numbers of 1.8–7.0 inclusive were grouped by Ehle as 3:1 segregations, while 7.2–41.0 were considered to be caused by duplicate factors.

TABLE 3. NUMERICAL PROPORTIONS, OBTAINED BY NILSSON-EHLE IN HIS CROSSES OF RED×WHITE WHEATS. (x:1)

1.8	2.0	2.0	2.8	2.9	classed as 3:1
3.0	3.0	3.2	3.3	3.3	
3.6	3.6	4.0	4.1	4.2	
5.2	5.3	6.0	6.0	6.5	
6.5	7.0				
7.2	8.4	8.7	12.0	13.0	classed as 15:1
13.5	13.7	14.0	16.0	16.0	
16.0	18.0	18.0	19.0	19.5	
21.5	22.0	24.0	24.0	26.0	
28.0	30.0	38.0	38.0	41.0	

This example has been followed by a great many other geneticists, so that at present each proportion, surpassing to a considerable degree the monohybrid ratio 3:1, is classed as 15:1 or 63:1 or a higher theoretical proportion, and is thus ascribed to duplicate, triple, or quadruple factors. And as these factors are taken to be wholly independent in their inheritance, they must be supposed, according to Morgan's view of the structure of chromosomes, to be localized in different chromosomes.

This consequence of the hypothesis of multiple factors seems to me not at all necessary. Agreeing with Morgan's supposition of the function of the chromosomes as warehouses of Mendelian factors, one is not obliged to assume the localization of these so-called multiple factors in different chromosomes. On this point Bateson's opinion (1926, p. 235) is quite right, when he says: "Having in view the various facts and considerations here enumerated, I think we shall do genetical science no disservice if we postpone acceptance of the chromosome theory in its many extensions and implications." The genetical building of hypotheses, constructed by Morgan and his staff, is such a fascinating force for all geneticists that it becomes a very difficult thing to withdraw from its influence and to keep an independent attitude towards these problems.

We must confess openly that at present nothing is known of the localization of these supposed multiple factors. It is true, attempts have been made by some authors to get some positive knowledge concerning this problem. If we should succeed in demonstrating as a fact that a certain Mendelian factor is linked to one member of a series of multiple factors, while it is inherited independently from the other members, we should have gained a strong indication in favor of the localization of these multiple factors in different chromosomes. But up to the present we have not succeeded.

Woodworth published in 1921 the results of his extensive studies on the inheritance of the color of cotyledons and of seed-coats in the soybean; he concluded therefrom that the yellow color of the cotyledons is caused by the presence of two factors *D* and *I*; they produce the same effect, as far as cotyledon color is concerned, whether one or both are present. On the other hand, green seed-coat proved to be dominant to yellow seed-coat and in F_2 a ratio of three green to one yellow was obtained. There is an apparent repulsion between green seed-coat and yellow cotyledon, and conversely between yellow seed-coat and green cotyledon. The evidence available indicated about 13 per cent crossing over, or a gametic ratio of 2:13. Woodworth assumed the localization of the two duplicate factors in different chromosomes and worked out in a latter paper (1923) the linkage-intensities, while he takes arbitrarily the relation to exist between one of the duplicate cotyledon-factors and the seed-coat-factor. In this supposition, however, there remain two weak points.

First, Terao had found in his experiments the inheritance of the color of cotyledons in the soybean to be wholly maternal, while the other characters studied did show an important Mendelian segregation, so that experimental errors seem to be excluded. How far these results of Terao may be brought into agreement with those of Woodworth cannot be judged now; the possibility remains that here a case is found, comparable to the double-throwing stocks, of the phenomena compiled by Bateson (1926) under the heading of anisogeny.

And secondly, Woodworth has assumed without delay that the duplicate factors for cotyledon colors are inherited independently, and that they are thus localized in different chrosomomes. His experiments have produced evidence only in favor of the conclusion that there exists a linkage between the factor for seed-coat color and those for the colors of the cotyledons, but his results can be explained as well on the assumption that both these factors are localized in the same chromosome. It is an interesting detail that the frequency-polygon in Woodworth's paper, showing the variations of segregating plants, grouped in the 15:1 class, possesses two peaks, one at 13:1, the other at 21:1.

We owe to Sax (1923) a second study which seemed to produce some evidence in favor of a linkage existing between a single factor and one of a series of multiple factors. The various seed weights of beans are inherited without any doubt; according to the present views they seem to give rise to the assumption of a series of multiple factors. Sax's researches led him to the conclusion that one of the members of this series was linked to the factor for pigmentation which is necessary for the development of any color in the seed-coat. If this were true, then strong evidence in favor of the localization of these multiple factors in different chromosomes would be obtained, but I think in my own studies on this problem (1925) to have proved that Sax's data are not conclusive because of the fact that the seed weight in beans depends upon two types of factors, which influence the seed weight in inverse directions. A number of accumulative multiple factors may be assumed to explain the increase of the weight of embryo contained within the seed-coat, while a single factor with an

inhibiting function is at work in the seed-coat itself and is opposed to the increasing development caused by the multiple embryonal factors. According to the data available, I think it is this inhibiting factor which is linked to the pigmentation-factor *P*, so that Sax's hypothesis cannot be supported.

This is the critical point in the problem: we can assume that so-called multiple factors are localized in the chromosomes, but all evidence is wanting in favor of the hypothesis that members of such a series are localized in *different* chromosomes. Can it now be accepted with certainty that crossing-over, or, as I should prefer to say, the exchange of factors between homologous chromosomes, is accomplished by means of the process of chiasmatypy only, or, in other words, by breaking one or two larger or small parts from the chromosomes with a union between these parts, according to the very attractive scheme of Morgan? If this were so, I could not see any other possibility to explain numerical proportions above 3:1, and I should be inclined to conclude, in the case of *Lamium*, mentioned above, that there are present 5 multiple factors and a linkage between two of them. But on this critical point there exists no certainty at all. In 1916 Goldschmidt clearly indicated the possibility of obtaining crossing-over without the chiasmatype theory; in 1924 McClung declares: "There is absolutely no evidence that they break and recombine as Janssen's chiasmatype theory demands" (1924, p. 658), and so long as such an important collection of facts can be compiled pleading against a monopoly of chiasmatypy, as Bateson (1926) presented in his interesting Joseph Leidy Memorial lecture, os long are we not only justified, but even obliged to call into question the correctness of this schematic suggestion.

There is, however, another interpretation for all numerical proportions above 3:1, a solution which does not reckon with multiple factors as independent corpuscles and which for its more physiological character deserves full attention and exhibits more attractiveness than the purely morphological conception of multiple factors.

Fig. 1

First by Cuénot, later by the school of Morgan and other geneticists, a phenomenon has been discovered that seems to be rather frequent in *Drosophila*: multiple allelomorphism. In the cases thus far ascribed to multiple allelomorphism, the differences between the various types can be described in qualitative terms; it seems, however, not at all excluded that quantitative differences may form the genotypic basis for these qualitatively varying phenotypes. How far this may be so in the classical series of eye colors in *Drosophila*, I cannot

say, but another simpler example can illustrate this as well. In the researches of the late R. P. Gregory (1923) on *Primula sinensis* full evidence can be found in favor of the supposition that the extension of the yellow eye in the center of the flower is caused by a series of multiple allelomorphs. "Primrose Queen" has an eye that covers half the petals; the original normal form is characterized by a smaller eye, enclosing nearly a quarter of the petals, while in "Queen Alexandra" the eye is almost absent. These multiple allelomorphs are considered by Bateson (1926 p. 205) to diverge by quantitative differences only: "W makes the whole petal white, as in Queen Alexandra; $W-\frac{1}{4}$ leaves the band of yellow in the normal; $W-\frac{1}{2}$ allows the extension over a further segment, as in Primrose Queen. So long as nothing lower than Primrose Queen is known, that may be taken as the basal negative."

Bateson extends this supposition to the case of the Dutch and the Himalayan rabbit, which form with the self-colored a multiple series. I may quote his own words here: "From whatever parentage the albino can be derived, it cannot, crossed with the Himalayan, produce a dark-eyed or a self-coloured form. On the other hand, the allelomorphism of self, Dutch and the albino, does not involve a multiple series. Dutch crossed with albino gives either Dutch again, or an approximately self-colored animal, according to the factorial composition of the albino. The nature of this distinction may be very simply expressed. We may describe the Dutch and the Himalayan as each wanting in some portion of the full complement needed to confer self-color, but the ingredient missing from the Dutch is not the same as that missing from the Himalayan. Nor are the factors for these two ingredients allelomorphic to each other. If we call C color and S the element conferring self-distribution, then the Dutch has C.S/2 and the albino c.S or c.S/2, the one capable of turning Dutch into self, the other leaving Dutch. The expression S/2 is not intended to mean the factor S is exactly halved in the Dutch. As in some similar examples, the fractionation of this factor is very indefinite. Probably no strain, even of thoroughbred Dutch, shows uniformity in correct marking, and as Castle has shown, there are many grades which have overlapping fluctuations. The Himalayan is deficient in another ingredient. For simplicity, we suppose this to be the C spoken of above. The C, C/2, and c form a multiple series; and the Himalayan bred to the Dutch has the power of making F_1 self, since, though having only half of C, it has the whole of S. Thus:

$$\text{Dutch } C\cdot\frac{S}{2}\times\text{Himalayan } \frac{C}{2}\cdot S$$

$$F_1 \text{ Self } C\frac{C}{2}\cdot S\frac{S}{2}$$

F_2	Self	Dutch	Himalayan	Dutch-Himalayan
	$C\cdot S$	$C\cdot\frac{S}{2}$	$\frac{C}{2}\cdot S$	$\frac{C}{2}\cdot\frac{S}{2}$

. Bateson did not offer any further development of this principle, but I think that he has produced here the embryonal form of a hypothesis, which can explain the phenomena thus far interpreted with the aid of multiple factors. We can

define this principle in these words: Factors, forming a series of multiple allelomorphs, are different not in qualitative, but in quantitative nature.

We may now take into consideration the results of Correns' studies (1919) on a variegated Shepherd's purse (*Capsella bursa pastoris*), and the conclusions drawn by him from his experiments. The data can be summarized as follows: The variegated race, *Capsella bursa pastoris albovariabilis*, inherits its variegation according to Mendel's laws; it is however not constant, but variable. By means of selection of more white or more green plants, or conformable branches of a plant as seed-parents, a change of the mean coloring in the offspring can be obtained; in one direction a constant green state is reached, in the other, however, probably owing to technical causes, a variegated form (containing much white) results, which can be kept in the same intensity by continuous selection in the same direction. As long as constancy (self-colored green) is not obtained, the selection can be exercised in both directions; the interstages are not fixed.

The theoretical interpretation is supposed by Correns to be the following: To take an analogous case one might suggest that to the material basis of the factor as a great molecule, the same group of atoms can be added many times, let us say ten times. This number is variable; it can increase or decrease under the influence of unknown circumstances, which are circumstances external to the factor. Each number of groups of atoms added to the molecule would represent a certain proportion between green and white on the plant. The difference between this interpretation and that of polymery (multiple factors) would be, then, that the state of this factor, that is, the number of groups of atoms, annexed to the molecule is a variable one, even during the ontogenetical development of the individuals. One or two of these states would be constant when all possible groups of atoms are added, or when all groups are wanting. The one state is realized by the homogeneous green, the other by the homogeneous white.

One can suppose here the existence of a number of multiple allelomorphs, which are unstable with two exceptions, discerned by quantitative differences and in their genetical behavior giving the same results as the assumed multiple factors in other cases.

We can cite another case, somewhat parallel to that of Correns, from the researches of East and myself on the inheritance of self-incompatibility or self-sterility. In both researches the F_1 grown from two self-incompatible parents showed a rather complicated behavior. Nearly all individuals were cross-compatible, but self-incompatible, or they behaved differently with regard to their compatibility towards their sibs; a clear classification into groups according to their behavior could not be obtained. Taking profit of the end-season fertility occurring in self-incompatible Nicotianas or applying continuous sib-mating in *Verbascum*, we both got later generations that consisted of two or four groups only. The constitution of these later generations can be explained rather safely by assuming the presence in the style of two oppositional factors, which are inhibitive to the growth of pollen tubes containing one of these factors. In my opinion the irregularities in the former generations indicate the presence of other multiple allelomorphs, which after the segregation gave origin to a number of

intermediate stages, from which the different behavior of the earlier generations can be derived. By continuous selfing or by continuous sib-mating these many multiple allelomorphs can be expelled, so that only two of them remain, which are wholly stable and do not show an exchange of their elements. The presence of these stable factors gives rise to the very regular appearance in later generations.

If we would try to explain these results by means of multiple factors, localized in different chromosomes, this would be possible only with the assumption that during the succeeding generations a great number of loss-mutations should occur, which can limit also the number of oppositional factors. But if we make this supposition, an unsolved problem remains: if these mutations by loss could occur with such great frequency, what is then the reason that this process of mutation stops immediately as soon as the number of factors is restrained to two, and why does this loss of factors not proceed until the oppositional factors are wholly discarded? This total loss must lead to self-compatibility or self-fertility, and this was never or only very exceptionally observed in East's and in my own experiments.

Now this is the line along which I should like to explain a number of phenomena thus far ascribed to multiple factors. We are justified in supposing that besides the chiasmatype there are also other processes of exchange between chromosomes during the reduction division or during other earlier divisions. We suggest that in both homologous chromosomes a pair of factors of different quantitative value is present; by exchange of their elements these factors can produce a number of factors of the same or other values. Or, to take the comparison Correns used; in each of the homologous chromosomes a molecule is present with one or more groups of atoms and these groups of atoms can be interchanged in both directions by both molecules. All these molecules with a number of groups of atoms form a series of multiple allelomorphs.

A table may serve to make clear the supposed state of things:

TABLE 4. X IS THE MOLECULE. 1, 2, 3, 4 ETC. ARE THE GROUPS OF ATOMS ADDED

Parents		F_1	Gametes of F_1	F_2-ratio	$F_1 \times R$-ratio
$\frac{X+1}{X+1}$	$\frac{X+0}{X+0}$	$\frac{X+1}{X+0}$	$X+1$ | $X+0$ 1 | 1	3:1	1:1
$\frac{X+2}{X+2}$	$\frac{X+0}{X+0}$	$\frac{X+2}{X+0}$	$X+2$ | $X+1$ | $X+0$ 1 | 2 | 1	15:1	3:1
$\frac{X+3}{X+3}$	$\frac{X+0}{X+0}$	$\frac{X+3}{X+0}$	$X+3$ | $X+2$ | $X+1$ | $X+0$ 1 | 3 | 3 | 1	63:1	7:1

In principle we can get thus the same numerical ratios as with the assumption of independent multiple factors.

Probably, however, this process will be complicated by the circumstance that certain numbers of groups of atoms possess more stability than others, so that deviations from the theoretical proportions are obtained. I must, however, confess to seeing no sharp interpretation for the proportions 13:1 and 21:1, as found in the back-cross of the peloric form of *Lamium*. There are two points to which I should like to draw your attention. First, these numbers (3:1; 7:1;

13:1; 21:1) seem to build a regular series, according to the formula $(X^2+X+1):1$ in which X can represent any entire number, 1, 2, 3, 4, etc. Secondly, it is a coincidence of possible importance that Woodworth in his F_2-generations for color of cotyledon in the soybean obtained also peaks at 13:1 and 21:1, as his frequency curve shows. And this especially, as it cannot be excluded that here a case of anisogeny occurs, that is, that as in the double-throwing stocks the pollen grains transmit only the recessive factor, while the egg cells belong to different genotypes. The results of Terao have produced some evidence in this direction, and if this may be true, the frequencies demonstrated by Woodworth as 13:1 and 21:1 would represent frequencies of gametes, as did the back-crosses in *Lamium*.

At present we do not possess any means of producing conclusive evidence for a decision as to whether two factors of a series are localized in the same or in different chromosomes, and this will thus remain a future task; but so long as in this matter no positive data are available, in my opinion both suggestions, that of independent multiple factors in different chromosomes and that of multiple allelomorphs of varying value in the same locus in the same chromosome, have just the same right in an interpretation of these phenomena, and I prefer the second supposition. I hope to have shown to you that there is great danger in accepting too readily the very attractive hypothesis of Morgan's monopoly of chiasmatypy and his morphological scheme of crossing-over, while another conception of more physiological nature can be defended as well. In such doubtful cases it will remain a matter of subjective sympathy what solution we may prefer, until in future a decisive experiment can be produced.

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PRELIMINARY EXPERIMENTS IN SELF- AND INTER-FERTILITY OF PISTACIA[1]

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Introduction

Sicily supplies the world's market with pistaches of the finest quality in respect to green color, size, and flavor of the kernel. The area containing pistache plantations in Sicily aggregates about 4,000 hectares with a yearly export of approximately 2,000 to 3,000 quintals of shelled kernels. The average annual yields per hectare are from one half to one quintal of shelled kernels. Production records examined by the author in the Duchy of Bronte and covering a period of thirty years, showed per decade only one good yield, three moderate yields, and six crop failures.

A survey of all the more important pistache plantings in Sicily was made during the years 1924–25 to determine the extent to which insufficient pollination might have a bearing on the situation, and it was found that the number of empty fruits per orchard amounted in some cases to 80 per cent of the total crop.

It seemed important to investigate this failure and to study its relation to other biological influences that very often prevent a good yield.

Pollen Germinability and Longevity

To determine the extent to which the longevity of pollen grains might be affected by conditions of temperature, and how, under laboratory conditions, maximum results might be obtained, pollen was held in different solutions and at different temperatures. Best germination of pollen was obtained at room temperatures of 20–25°C. and in an aqueous solution of cane sugar (saccharose) containing from 15 to 18 grams of sugar dissolved in 100 cc. of water; other sugar solutions, especially milk sugar (lactose), gave inferior results.

To more closely approximate the stigmatic secretions, a nutritive solution of honey in water was prepared and used apparently for the first time in studies on pollen germination. Honey freshly obtained from a bee hive was pasteurized and then diluted with water so as to make a solution containing 12 per cent of honey.

Under optimum laboratory conditions for pollen germination, the following averages were obtained for the species studied:

Pistacia terebinthus, 67 per cent; *P. vera*, 36 per cent. *P. hybrida* is divided into two groups in the matter of pollen germination, depending upon the individual

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plants from which the pollen is obtained. One group gives a high percentage of pollen germination, 47 to 52 per cent, and the other gives a much lower value, 27 to 31 per cent. The longevity of the pollen of these varieties, according to daily determinations, was from 15 to 20 days for pollen collected shortly before the opening of the anthers and kept in the laboratory at temperatures varying from 20 to 25°C. When pollen from these same sources was placed in Petri dishes out of doors, the power of germination was lost after two days under shade conditions, and after a few hours when exposed to the sun.

Artificial Rain During Stigmatic Receptivity

Some seasons continuous and persistent rains with high temperatures prevail causing a development of flowers but a complete failure in the setting of the fruit. To determine the extent to which the rain is responsible two plants, one staminate and another pistillate, were put in the same cage and covered with unbleached cotton to obtain an artificial damp chamber. Every two hours water was sprayed against the top of the cage to imitate the action of the rain, and this was continued until the end of the blooming period. The setting of the fruit was a complete failure, with the exception of a few shells without kernels. The effect was not as pronounced when water was sprayed on plants in the same manner.

From these experiments we may say that humid conditions more than the rain are responsible for a crop failure.

Traditional Artificial Pollination

For centuries the pistache in Sicily was pollinated by shaking branches of staminate plants on pistillate plants as Bocconi described it in "Flora Sicula" about a century ago. Later, pollination was obtained by suspending in the center of the pistillate plant a branch of the staminate, with a cord made from leaves of a palm (*Chamaerops humilis*), a few hours before the dehiscence of the anthers; a method that is similar to the old practice of caprification of figs or the Arab pollination of the date palm. The extremities of the branches were protected with clay to prevent excessive loss of water during the heat of the day. The branches were changed after a few days when the flowers were completely dry.

Actually no one of the preceding methods is used in Sicily and in fact in some localities are completely unknown. The pollination now is made in the natural way; in some places the plan is used, as I found in Trapani, of budding a branch of the pistillate plant with the staminate, but the method is completely impractical because the pistache is a dimorphic plant, and after a few years the staminate plant through its superior energy of growth largely absorbs the pistillate, resulting in a considerable diminution of the crop.

The Value of Some Staminate Pistacia Species as Pollenizers for Pistacia Vera

It has long been the customary belief of pistache growers in Sicily that *P. terebinthus* constitutes the natural, and therefore the more desirable pollenizer for the commercial varieties of *P. vera*. It was common practice, therefore, when

top-working wild terebinthus trees to leave the staminate individuals as pollenizers. It gradually became evident, however, that the staminate varieties of *P. vera* were superior in pollination efficiency to those of *P. terebinthus*. Nevertheless, the latter is still largely used as the only source of pollen in many localities, and to supplement *P. vera* in others. In order definitely to determine the relative value of pollen of these species, experiments were conducted using both pollens, and also those of two groups of hybrids, on pistillate trees of *P. vera* and *P. hybrida*.

Pistillate flower clusters for this purpose were inclosed within paper bags and sealed with paraffin over the seams and about the openings. To make doubly certain that no foreign pollen might enter, two sacks were used, one of smaller size within a larger outer sack. Careful note was made of the progress of the development of the stigmas, and pollen was applied precisely when the latter were apparently most receptive. All the doubtful flowers, as far as receptivity is concerned, were cut out with sharp scissors before the pollination. The results of these pollen applications are shown in table 1.

TABLE 1. SHOWING THE RESULTS OF HAND POLLINATION OF PISTACIA VERA WITH POLLEN FROM PISTACIA TEREBINTHUS, TWO FORMS OF HYBRIDS, AND P. VERA

Pollen Used	No. of fl. clusters pollinated	Total No. of nuts	Per cent normal nuts	Per cent half empty nuts	Per cent empty nuts	Per cent defective nuts
P. terebinthus	91	1075	27	20	53	73
P. hybrida, Gasp. (1)	37	357	(31)	9	40	49
P. hybrida, Gasp. (2)	30	460	17	7	76	85
P. vera	70	960	65	12	23	35

Of the four forms of pollen used, the highest percentage of normal nuts was obtained with that of *P. vera* (65 per cent). Pollen of the hybrid of Group I gave the second highest percentage (31 per cent), while that of *P. terebinthus* was third in order (27 per cent) with a percentage of less than half that of *P. vera*. Pollen of the second group of hybrids gave the lowest percentage (17 per cent) of any of the four tried.

The Value of Different Pollens on Pistacia Hybrida

The set of *P. hybrida* (1) when artificially self and inter-pollinated is shown in the following, table 2:

TABLE 2. PISTACIA HYBRIDA (I) SELF AND INTERPOLLINATED

Pollen Used	No. of clusters pollinated	No. of nuts obtained	Nuts with seeds Per cent	Empty nuts Per cent
P. terebinthus	41	2280	45	35
P. vera	23	1528	37	63
P. hybrida (1)	51	2448	58	42

P. hybrida (1) has a greater affinity for its own pollen than for that of either parent species, *Pistacia terebinthus* and *P. vera*.

Extent and Frequency of Non-Development of Pistache Kernels

Failure of the pistache to develop plump kernels is not peculiar to the cultivated varieties, as it occurs in all other species of the genus. In order to determine its frequency in cultivated plantings, seeds were obtained from healthy trees known to be within range of pollination by staminate trees, and examined, with the result shown in the following table of which I will give only a summary:

TABLE 3. PERCENTAGE OF EMPTY FRUITS IN PISTACIA

Species Examined	Origin of fruit	Quantity in gms.	Empty fruits Per cent	Observations
Pistacia vera, L.	Malta	20	53	
Pistacia vera, L.	Catollica Eraclea	5550	10	Harvest 1924
Pistacia vera, L.	Catollica Eraclea	2500	12	Harvest 1925
Pistacia vera, L.	Acireale	7200	37	
Pistacia hybrida, G.	Malta	20	46	
Pistacia hybrida, G.	Acireale	500	54	
P. terebinthus L.	Malta	20	59	
P. terebinthus, L.	Acireale	2300	47	
P. terebinthus, L.	Acireale	1800	67	
P. chinensis, B.	Washington	74	—	Selected fruit with kernels
P. atlantica, Desf.	Tripoli	2400	29	
P. integerrima, Stew.	Lahore (India)	450	41	
P. lentiscus, L.	Malta	20	27	

From these figures it will be seen that in the present commercial *P. vera* plantings the percentage of nuts without kernels ranges from 10 to 53 per cent while that of the two forms of hybrids ranges from 46 to 54 per cent and that of wild species from 29 to 67 per cent.

Dependence of the Pistache upon Pollen

In an experiment to determine definitely whether the kernel effectiveness, in so far as commercial orchards were concerned, was due to a lack of pollen or not, clusters of *P. vera* flowers were inclosed within paper sacks at pollination time, in order to exclude pollen entirely, and subsequent count was made of the percentage of nuts developed.

The work was conducted at two points, Cattolica Eraclea and Acireale. The number of nuts which set was found to vary greatly with individuals and plants, but corresponds almost exactly both within and without the sacks. Those inclosed were entirely without kernels, whereas those on the outside developed the customary percentage of both filled and empty nuts. Those on the outside however were artificially pollinated.

Early, Intermediate, and Late Flowers

It has been recorded by other investigators that the cultivated pistache is proterandrous. This was found also by the writer to be true, but in less degree with *P. terebinthus* and certain other species.

On several excursions I collected several natural hybrids between *P. vera*

and *P. terebinthus*, in the forests of terebinthus in Sicily, and some of these were budded in a commercial orchard, the first important planting in Sicily, near Cattolica Eraclea in the province of Girgenti.

In this orchard now there are present about 700 staminate plants and 4,000 pistillate plants and the blossoming takes place in different individuals at different times to cover completely the period of stigmatic receptivity with fertile pollen.

Ovular Abortion

The hand pollinated clusters give an appreciable percentage of empty nuts, even when all flowers showing insufficient stigmatic secretion are removed.

In the artificial pollination experiments it has never been possible to eliminate completely this small percentage of empty nuts, which does not exceed 5 or 6 per cent of the total set.

This behavior may be explained by an ovular abortion that takes place after fecundation is completed. The ovular abortion must not be confused with incomplete development of the kernel, which often occurs when the season is very dry, especially in the volcanic rocks of Mount Etna.

The action of sunlight on dark rocks, resulting in an intense radiation of heat, so affects the plants in certain dry years that the development of the kernel inside the shell is arrested for as long as three months after the shell itself has attained full size. The kernel in such cases cannot acquire full size in the little time that remains after adequate moisture is supplied, and as a result a large percentage of defective fruits are harvested that are known to the commercial trade as half-empty nuts.

Conclusions

1. Pistache as a wind-pollinated plant is subject to great loss of pollen. Moreover, the brief duration of germinability of the pollen is due to such natural out-door conditions as the action of sunlight—preventing, as it does in a few hours, normal germination—and to other appreciable factors, such as humidity, precipitation, and temperature.

2. The pollen of *Pistacia terebinthus*, used for centuries as a natural pollinator of *Pistacia vera*, shows from pollen germination tests and artificial pollination a small affinity for pistache when compared with that of some hybrids and with that of the natural pollenizer.

3. The proterandrous characteristic of the genus *Pistacia* is one of the factors affecting the high percentage of empty nuts, because a great deal of pollen is not potent when the stigmas are receptive.

4. The genus *Pistacia* can produce nuts without kernels in complete absence of pollen. In the commercial plantings of pistache the cause of empty nuts must be attributed to the absence of fertile pollen, rather than to an abortion of the ovules or to adverse meteorologic conditions.

5. The productive efficiency depends much on different ecological factors and is in direct proportion to the numbers of staminate plants present in an orchard,

to the best location of these plants with relation to the winds dominant during pollination, and to different types of staminate hybrids, in order to obtain continuous production of pollen during a period of about 40 days.

6. The staminate hybrids collected by the author during several excursions into forests of *Pistacia terebinthus* in Sicily have a special biological function so far as concerns pollination, for they furnish good pollen, in the full period of stigmatic receptivity, when the natural pollenizer is inactive. A secondary function of the late staminate hybrids is to furnish pollen in order to insure pollination, when a frost might destroy the first flowers of *Pistacia vera*.

7. The value of those hybrids must always be determined by pollen germination tests, artificial pollination, and by keeping special records of the length of the blossoming period in relation to the stigmatic receptivity.

8. No definite statement can be made now relative to the choice of hybrids in commercial plantings. It seems to me advisable, from these preliminary experiments, to select the largest possible number of hybrids, because every year we must attribute a special value to a hybrid.

9. The new plantations must have instead of one staminate to forty pistillate plants, one to six, or one to four. One half of the staminate plants must be hybrids, alternately distributed with the regular staminate plants throughout the orchard.

10. I hope with the assistance of Dr. Swingle (of the U.S. Department of Agriculture) and of the International Education Board (Rockefeller Foundation) to conduct further investigations on this subject next year while in California.

UNTERSUCHUNGEN ÜBER DIE CYTOLOGIE PFLANZLICHER SPECIES-BASTARDE MIT GLEICHEN CHROMOSOMEN-ZAHLEN DER ELTERN[1]

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In meiner Zusammenstellung der Chromosomen-Zahlen für die "Tabulae biologicae" (1926) habe ich soeben alle mir bekannt gewordenen Fälle von pflanzlichen Bastarden näher aufgeführt, die bisher in bezug auf ihre Gametenbildung cytologisch untersucht wurden. Sie können in zwei Gruppen gegliedert werden, je nachdem die Eltern ungleiche oder gleiche Chromosomenzahlen haben. Bei ersteren sind naturgemäss mannigfache Unregelmässigkeiten in der Reduktionsteilung zu erwarten, die sich aus dem veränderten cellulären Balancement ergeben. Das kennen wir bereits von Rosenbergs ('03, '04, '09) klassisch gewordenem Bastard *Drosera rotundifolia*×*longifolia*, und das wurde an vielen anderen Species, die später untersucht wurden, immer wieder neu bestätigt. Hier sehen wir besonders oft Störungen in der Paarung, Spaltung und Verteilung der Chromosomen während der meiotischen Mitosen; hier finden wir die Spindel unregelmässig entwickelt, und wir sind versucht, mit Correns ('01) bereits den plasmatischen "Entfaltungsapparat" der Keimanlagen gestört zu denken, freilich einen Apparat, der wohl vorher von der Kernsubstanz aus abnorm beeinflusst wurde.

Aber von all diesen interessanten Hybriden wollen wir heute nicht sprechen, umsomehr als Renner ('24) jüngst in seinem grossen Sammelreferat gerade diese Störungen eingehend erörterte, und ich selbst (Tischler '25) für die "Bibliographia Genetica" eine Zusammenfassung unserer diesbezüglichen Kenntnisse brachte. Der Gegenstand unserer Ausführungen soll vielmehr eine Untersuchung über das Verhalten solcher Bastarde sein, welche gleichchromosomige Eltern haben. Renner hat freilich durchaus darin Recht, dass die Chromosomenzahlen an sich für die Fertilität oder Sterilität eines Hybriden gleichgültig sind. Aber der celluläre Mechanismus wird in den Fällen "überzähliger" Chromosomen eines Elters doch meist sofort ein anderer als in den Fällen "balancierter" Zahlen.

Stehen bei gleichchromosomigen Eltern diese dazu noch systematisch einander nahe, und das bedeutet vorläufig immer in erster Linie das Vorhandensein einer grossen morphologischen Aehnlichkeit, so pflegen die F_1—, wie die folgenden Generationen der Bastarde fertil zu sein, nicht durchweg so fruchtbar wie die Eltern, aber doch mit immer noch relativ günstigem Procentsatz an

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[1] Presented before the International Congress of Plant Sciences, Section of Genetics, Ithaca, New York. Aug. 18, 1926.

tauglichen Gameten. In diesen haben wir uns die zu einander harmonischen Chromosomensätze und Gene zu denken. Je nach den Aussenbedingungen schwankt der Bestand an tauglichen Pollenkörnern und Embryosäcken, ja es wäre denkbar (Michaelis '26), dass die Bastarde *an sich* darin empfindlicher sind als die Eltern, aber die Hauptsache ist die principielle Möglichkeit einer Weiterzucht in den folgenden Generationen. Cytologisch sind uns Bastarde dieser Kategorie von *Salix*, *Melandrium*, *Cardamine*, *Saxifraga*, *Crataegus*, *Rubus*, *Fragaria*, *Potentilla*, *Geum*, *Alchemilla*, *Rosa*, *Prunus*, *Phaseolus*, *Citrus*, *Primula*, *Gossypium*, *Oenothera*, wohl auch von *Xanthium*, ferner von *Triticum* und *Orchis* bekannt.

Bereits durch solche verhältnismässig gering erscheinende Eingriffe in den cellulären Mechanismus, wie sie etwa das Auftreten eines "überzähligen" Chromosoms mit sich bringt, steigt der Procentsatz an untauglichen Geschlechtszellen. Es sei z. B. auf die soeben erschienene Abhandlung von Blakeslee und Cartledge ('26) verwiesen. Hier bleibt doch wenigstens der sonst fertil machende Chromosomensatz *in toto* beisammen. Die Sterilität muss natürlich weit höher werden, wenn zwei Eltern gekreuzt wurden, deren ganze Chromosomensätze unharmonisch zu einander bleiben. Das wird im allgemeinen der Fall bei Species sein, die auch systematisch sich ferner stehen, bei denen also phaenotypisch die Unterschiede im Genbestand der Organismen sich stark auswirken. Bei der Gametenbildung solcher total steriler oder doch fast steriler Hybriden verhalten sich die einzelnen verschieden. Wir können unterscheiden:

(1) Die Reduktionsteilung ist annähernd normal, erst nachträglich setzt die Degeneration der Tetraden ein [*Mirabilis Jalapa*×*tubiflora*—Tischler '08, *Prunus Besseyi*×(*Munsoniana*×*triflora*)—Dorsey '19, *Viola odorata*×*hirta*—Schnarf '22, *Epilobium hirsutum*×*montanum*—Håkansson '24, *Epilobium roseum*×*parviflorum*—Schwemmle '24].

(2) Regelmässige und unregelmässige Reduktionsteilungen kommen unmittelbar neben einander vor (*Ribes sanguineum*×*aureum*—Tischler '06, *Godetia amoena*×*Whitneyi*—Håkansson '25, *Bryonia alba*×*dioica*—Tischler '06a).

(3) Die Reduktionsteilung ist so unregelmässig, dass normal aussehende Gameten überhaupt nicht mehr gebildet werden (*Raphanus sativus*×*Brassica oleracea*—Karpetschenko '25, *Epilobium parviflorum*×*roseum*, wenigstens bei der Teilung der Embryosack-Mutterzelle—Schwemmle '24, *Primula floribunda* ×*verticillata* und reciprok—Digby '12).

Zahlreiche andere sterile Bastarde, vor allem unter den polyploiden Specieskreuzungen der oben genannten Rosaceen-Gattungen, sind weiter in der Literatur angegeben. Eine genauere Analyse steht wohl fast überall noch aus. Und die Unfruchtbarkeit der beiden von Babcock und Mann-Lesley ('26) beschriebenen Bastarde *Crepis setosa*×*Dioscoridis* und *Crepis setosa*×*tectorum* ist mir nur aus den starken Grössendifferenzen der "homologen" Chromosomensätze wahrscheinlich. Einzig in dieser Publication sind die elterlichen Chromosomen durch Messungen in ihrer specifischen Grösse festgelegt und in dem Satze des Bastards identificiert.

Hier wollen wir auch mit unseren eigenen neuen Untersuchungen anknüpfen. Ich habe erneut das Studium des Bastards *Ribes sanguineum*×*aureum* aufgenommen. Vor zwei Jahrzehnten, als ich eine Schilderung der Pollenentwicklung dieser Pflanze gab, hatte ich z. B. die Eltern-Cytologie noch gar nicht berücksichtigt. Dieser hybride *Ribes* ist darum besonders interessant, weil er nur ein einziges Mal hergestellt und seitdem immer durch Stecklinge vermehrt wurde. Nach de Janczewski ('07), dem Monographen der Gattung, hat ihn der Gärtner Beaton in Strubland-Park (England) erzeugt; auch de Vries betont in seiner Mutationstheorie ('03), dass niemals eine Wiederholung der Kreuzung gelang. Dabei haben wir hier einen Fall, in dem nach morphologischer Betrachtung die Eltern ziemlich nahe zu stehen scheinen. Ich habe seiner Zeit mit de Vries mehr das Trennende als das Verbindende betont. Aber de Janczewski stellt in seiner eben erwähnten schönen Monographie wohl mit mehr Recht die beiden Eltern-Arten in zwei Nachbar-Sectionen, die sich nur durch die Form des Fruchtblattes und das Vorkommen der Drüsenhaare von einander sondern. Die sonstigen Unterschiede beziehen sich auf die Haltung der Zweige, auf die Blütenfarbe und gewisse Grössenverhältnisse bei Blättern und Receptakulis. Ferner wies aber de Janczewski darauf hin, dass in den cultivierten Rassen von *R. sanguineum* eine starke Tendenz zur Blütenfüllung sich bemerkbar macht. Dadurch könnte dann correlativ eine Schwächung der Sexualorgane resultieren, sodass die Sterilität mehr durch Aussen- als durch Innenfaktoren (vom Standpunkt der Sporenmutterzellen aus) bedingt wäre. Ich wollte sehen, ob cytologische Analyse hier den Fragecomplex würde etwas aufklären können.

Schon vor 23 Jahren stellte ich (Tischler '03) fest, dass die Eltern unseres Bastardes einen völlig normalen 8-kernigen Embryosack haben, und dass demgegenüber der Bastard-Embryosack total zu obliterieren pflegt. Aber ich sah auch, dass, wenngleich selten genug, ein normaler Embryosack mit Eizelle, Synergiden, Polkernen und Antipoden vorkommen kann, die Makrospore also damit zu einem mindestens gesund aussehenden Gametophyten ausgekeimt sein muss. Weder damals, noch 1906, noch jetzt nach erneutem Studium habe ich im Gegensatz dazu auch nur ein einziges auskeimendes Pollenkorn beobachtet (s. a. de Janczewski '04, '07, '08). In 5% Zuckerlösung, in der der Elternpollen gut auskeimt, erwies sich der Gesamtpollen des Bastardes als total steril. Meist war er auch völlig taub, ganz selten fand sich ein Korn von der Normalgrösse des *R. sanguineum* oder *R. aureum* ein. Ueber 30μ Durchmesser kam keines hinaus, die allermeisten waren noch viel kleiner.

Eine cytologische Untersuchung ergab heuer wie '06, dass zwar Unregelmässigkeiten bei der Reduktionsteilung vorkommen können und dass dann auch "überzähliger" Pollen im Tetradenverbande auftritt, dessen Kerngrösse sehr von der Norm abweicht, dass aber sehr vielfach die meiotischen Teilungen ungestört zu Ende geführt werden und der Mechanismus der Chromosomenverteilung nicht grob verändert ist.

Die Reduktionsteilung der Eltern war mir bisher in den Einzelheiten noch unbekannt geblieben. Ohne weiteres ergab sich jetzt, in erster Linie bei Betrachtung der Metaphasen, dass 8 haploide Chromosomen vorhanden waren. Diese

Zahl hatte ich s.Zt. auch für den Bastard bestimmt, aber bei nachträglicher Durchsicht der alten Präparate für meine Karyologie ('21-'22) vorübergehend aufgegeben. Der Grund dazu war der, dass ich die Spindeln mit den höchsten Zahlen als massgebend betrachtete, dabei aber nicht bedacht hatte, dass ich dann solche Präparate als "Norm" ansah, in denen die Längsspaltung der Chromosomen bereits vollzogen und die Wanderung nach den beiden Polen eine etwas schleppende war. Die elterlichen Chromosomen erwiesen sich als deutlich verschieden gross, die von *R. aureum* waren im gleichen Stadium sichtlich kleiner und schmaler als die von *R. sanguineum*. Die Fixierung war bei beiden Species in gleicher weise mit Carnoys Flüssigkeit vorgenommen. Trotzdem war die Grössendifferenz etwa so gross wie in den beiden Rassen von *Phragmites*, die ich vor einigen Jahren cytologisch studierte (Tischler '18). Dieser Unterschied wirkte sich auf die Grösse der Kerne im jungen Pollen aus. Bei *Ribes aureum* waren nach 25 Messungen die sehr gleichmässigen Nuclei im Mittel 4.12μ, bei *R. sanguineum* im Mittel 6.17μ breit. Der Gegensatz erschien so frappant, dass ich bei *R. sanguineum* auch noch Kerne mass, welche etwas jüngeren Tetraden angehörten. Der gemeinsame Plasmakörper erschien hier noch in der Mitte zusammenhängend, und die streifenförmigen Verbindungsfasern der Kerne waren in ihren Resten zu sehen. In diesen deutlich jüngeren Stadien fand ich die Nuclei noch beträchlich grösser als die älteren von *R. aureum*, das arithmetische Mittel bei wieder 25 Messungen war hier 5.33μ. Die Grösse des jungen Bastardpollens, solange er noch im Tetraden-Verband zusammenhing, passte gut zu den obigen Zahlen. Natürlich berücksichtigte ich dabei nur solchen, der anscheinend eine normale Tetradenteilung durchgemacht hatte, bei dem also die 4 Körner annähernd gleichgross waren. Bei 25 Messungen erhielt ich das Mittel von 4.85μ; das arithmetische Mittel zwischen den Massen der gleich weit entwickelten Elternpollen wäre 5.15μ gewesen.

Aus der Arbeit von Santos ('24, p. 361) wissen wir, dass starke während der Entwicklung hervortretende Grössenunterschiede der Kerne in ihrer Auswirkung auf die Zellgrösse sich wieder verwischen können. So ist auch bei *Ribes* der reife keimende Pollen in seinen grössten Körnern ziemlich gleich gross. Ich mass für die Pollenkörner von *R. aureum* nämlich 39.2μ, von *R. sanguineum* 41.3μ.

Für unseren *Ribes*-Bastard möchte ich trotzdem die auch morphologisch ausgedrückte Disharmonie der beiderelterlichen Chromosomen zur Erklärung der Totalsterilität heranziehen. Natürlich wird es nun die nächste Aufgabe sein müssen, die beiden Sorten von Chromosomen auch innerhalb des Bastards selbst nachzuweisen. Aus Mangel an geeignetem Material kann ich im Augenblick diese Aufgabe noch nicht lösen. Ich hoffe, im nächsten Jahre hierüber genauere Auskunft geben zu können. Meine alten Präparate von 1906 zeigen mir bereits jetzt stärkere Grössendifferenzen unter den Chromosomen. Auch das Studium der somatischen Mitosen müsste ja die beiden verschiedenen Sätze zeigen. Wurzelspitzen in günstigem Entwicklungsstadium zeigen mir schon heute, wie hier einzelne "Individuen" unter den Chromosomen hervortreten, besonders ein Paar scheint beträchtlich länger als die anderen zu

sein. Ob sich aber eine so reinliche Scheidung der elterlichen Anteile vornehmen lässt wie bei den *Crepis*-Hybriden von Babcock und Mann-Lesley, erscheint zweifelhaft.

Die Gattung *Ribes* gehört nach meinen bisherigen Erfahrungen zu denjenigen, bei denen innerhalb der verschiedenen Sectionen eine bemerkenswerte Uebereinstimmung der Chromosomen-Zahlen geblieben ist. Aus 5 der 6 Subgenera de Janczewskis ('07) liegen Zählungen vor. Ich selbst habe mit Unterstützung meines Assistenten Dr. Jaretzky die 8-Zahl der Chromosomen festgestellt für Subgenus Ribesia (*R. petraeum* u. *rubrum*), Subgenus Coreosma (*R. sanguineum*, *R. aureum*, *R. nigrum*), Subgenus Grossularioides (*R. lacustre*), Subgenus Grossularia (*R. Grossularia*). Dazu kommt noch der Fund von Meurman ('25) bezüglich des Subgenus Berisia (*R. alpinum*). Die Studien sollen fortgesetzt und erweitert werden. Himmelbaurs ('12) Zählung, wonach *R. pallidum* (*R. rubrum*×*petraeum*) 10 Chromosomen habe, passt jedenfalls nicht gut zu unseren bisherigen Funden und ist vielleicht durch nicht vollständige Bindungen zu erklären.

Gerade in der letzten Zeit ist mehrfach gezeigt worden, dass nun selbst bei ganz sterilen Hybriden plötzlich Fertilität, ja selbst eine damit verbundene Constanz in den Folge-Generationen einsetzen kann. Das geht, soweit wir bis jetzt wissen, immer auf dem Wege der Chromosomenverdopplung vor sich. Von den oben genannten Bastarden ist das besonders lange für die Primelkreuzung (=*Pr. Kewensis*) bekannt. Aus mündlichen Angaben von Collegen Karpetschenko weiss ich, dass es auch für *Raphanus*×*Brassica* gilt. Und schliesslich hat Bleier ('26) es für *Aegilops ovata*×*Triticum dicoccoides* resp. *Tr. durum* nachgewiesen. Ferner sei an die Fälle erinnert, in denen die Eltern ungleiche Chromosomen-Zahlen haben: nämlich *Rosa Wilsonii* (Blackburn u. Harrison '24), sowie *Nicotiana glutinosa*×*Tabacum* (Clausen u. Goodspeed '25). In Analogie dazu ist zu erwarten, dass entsprechend Winges ('17) alter Hypothese sich die Polyploidie vieler Species erklären lassen wird.

Ohne eine derartige Vermehrung der Chromosomenzahl ist kaum ein Fall von ausgesprochener Fertilität bekannt, sofern die Eltern sich systematisch wenig nahe stehen. Der Fall von *Zea Mays*×*Euchlaena mexicana* (Longley '24), scheidet wohl dabei aus. Die bekannte Hypothese über den Mais-Ursprung zeigt jedenfalls, dass hier einer der Fälle vorliegt, in dem trotz grosser morphologischer Unterschiede genotypisch kaum stärkere Differenzen wahrscheinlich sind (vgl. noch besonders Kuwada '19).

Das müsste uns nun consequent zu dem Standpunkt führen, wie wir das in der Genetik schon allgemein tun, die phaenotypisch sich offenbarenden systematisch allein verwandten Merkmale als die unwichtigeren zurücktreten zu lassen. Wir müssten also eine Systematik anstreben möglichst losgelöst von all dem, was heute noch zumeist das Interesse des Systematikers ausmacht. Solch eine Systematik haben wir nun angebahnt erhalten durch die Verwendung der Serologie, wie sie die rasch bekannt gewordenen umfassenden Arbeiten der Schule von Mez (s. z. B. '24) uns aufzeigen. Ein Hauptergebnis dieser Studien ist das, dass im allgemeinen die "Eiweiss-Differenzierung" in dem

Masse zunimmt, in dem die betreffende Species einer Familie angehört, die an einem "Endzweige" des Stammbaums sich befindet. (Man denke auch an Rosens, '25, "complicatorisches Princip".) Je näher der Basis des Stammes, desto mehr stimmen auch Gruppen im Eiweiss überin, die nach morphologischer Betrachtung sehr verschieden sind. Die Regel ist vorläufig nur *cum grano salis* zu verstehen. Denn die Entwicklung braucht ja bei den Familien an der Basis nicht stehen zu bleiben. Aber es lohnt sich vielleicht, einmal zu untersuchen, wo die sterilen Arthybriden sich befinden, deren Unfruchtbarkeit doch mindestens zum grössten Teile auf unharmonische Gene und damit wohl verbundene Eiweiss-Differenzierung zurückzuführen ist. Ziehen wir Fockes ('81) Buch zu Rate, so handelt es sich in erster Linie um Bastarde von *Papaver*, *Viola*, *Verbascum* und *Digitalis*, dazu noch von *Rhododendron*, *Epilobium*, *Cereus* und *Hymenocallis*. Aus unserer oben aufgeführten Liste kämen weiter *Mirabilis*, *Raphanus*, *Prunus*, *Ribes*, *Godetia*, *Primula*, *Bryonia*, *Crepis* in Betracht. Fast alle Gattungen gehören aber zu Familien mit starker Eiweiss-Differenzierung, da sie als phylogenetisch junge von der Serologie angesprochen werden.

Das entgegengesetzte Verhalten, Fertilität bei morphologisch entfernt stehenden Eltern und gleichzeitigen Unterschieden in vielen Genen (was für den vorhin genannten *Zea-Euchlaena*-Bastard wohl nicht zuträfe), werden wir vielleicht am ersten innerhalb der an der Basis des Stammbaums stehenden Angiospermen-Familien, also z. B. der Ranales, finden. In diesem Zusammenhange sei zunächst wieder auf die teilweise fertilen Magnolien-Bastarde unserer Gärten verwiesen, die dringend eine moderne cytologische Bearbeitung verdienen (Tischler '25, p. 40). Aus gleichen Gedankengängen heraus beabsichtige ich in den nächsten Jahren besonders die Familie der Berberidaceen zu studieren. In erster Linie interessieren mich hier die Chromosomen-Zahlen und Formen der verschiedenen monotypen Gattungen einerseits, der artreichen andererseits. Seit langem sind aus dieser Familie zwei Hybride zwischen morphologisch sehr entfernt stehenden Eltern bekannt, die beide nur ein einziges Mal entstanden sind und darin wohl schon auch eine tatsächlich entferntere Verwandtschaft wahrscheinlich machen. Irgend eine anfängliche Hemmung muss existieren, die der leichten Kreuzbarkeit in den Weg tritt.

War diese aber einmal vom Zufall überwunden, so zeigten sich die beiderseitigen Kern- und Cytoplasma-Substanzen relativ harmonisch zu einander. Es handelt sich um *Mahonia aquifolium* × *Berberis vulgaris purpurea* = *Berberis Neuberti* und um *B. empetrifolia* × *Darwinii* = *B. stenophylla*. Der erstere Bastard entstand 1854 zu Bollweiler im Elsass und ist von da durch Stecklinge in alle botanischen Gärten übergegangen. Zwar ist er völlig blütenlos. Aber das spricht hier nicht gegen unser Raisonnement. Denn es muss bei den grossen Differenzen der Eltern überhaupt als Wunder erscheinen, dass der Bastard lebensfähig ist und nicht schon auf so frühem Stadium abstarb, wie wir das beispielsweise für *Iris Pseud-Acorus* (Sawyer '25) oder die *Epilobium* × *Chamaenerion*-Kreuzungen (Michaelis '25) kennen. (Vgl. Tischler '25, p. 40).

Fast noch interessanter ist der zweite Bastard, *Berberis stenophylla*. Lindley berichtet zuerst über ihn in Gardeners Chronicle 1864, und wir erfahren, dass Fisher und Holmes in Handsworth-Sheffield ihn hergestellt haben. (S. z. B. Hurst 1900.) Seit langem ist bekannt, dass der Bastard in wechselndem Masse fertil ist und dass selbst F_2-Individuen in grosser Menge aufgezogen sind. Als ich das Fertilitätsproblem dieses Hybriden jetzt aufgriff, waren mir nur die älteren Angaben von Hurst (1900) bekannt, nach denen zu 90% absolute Constanz in F_2 herrschen sollte, und nur 10% genau analysierte Abweicher nach den beiden Eltern schlagen. Dies und das Auffinden einer Anzahl Bilder, in denen ich eine grössere Zahl von Chromosomen zählte, als nach den Chromosomenzahlen der Eltern zu erwarten war, legten den Gedanken nahe, dass wir auch hier einen Fall der Chromosomen-Verdopplung vor uns hatten, die Fertilität im Gefolge hat. Die genaue Untersuchung bestätigte jedoch diese Vermutung ganz und gar nicht. Die scheinbare Erhöhung der Chromosomenzahl war auf schräg geschnittene frühe Anaphasen zurückzuführen. Und ich lernte in *Berberis stenophylla* einen Typ kennen, der sich durchaus im Sinne unserer eben skizzierten phylogenetischen Gedankengänge verwerten lässt.

Betrachten wir zunächst die beiden Eltern. *Berberis empetrifolia* gehört nach dem Monographen der Gattung C. K. Schneider ('05) zu seiner Sectio (1) Buxifoliae, *B. Darwinii* zur Sectio (15) Ilicaefoliae. Erstere stellt einen schlaffen, niederliegenden Strauch, letztere einen kräftigen, aufrechten Busch dar. Auch in Behaarung, Blattdornbildung, Blattform und Consistenz, in Blütenbildung und Blütenzahl, in der Grösse der Blütenteile wie in der Zahl der Samenanlagen sind weitgehende Differenzen vorhanden.

Beide Eltern haben nach meinen (mir grösstenteils von Herrn Dr. Jaretzky angefertigten) Präparaten 14 haploide Chromosomen. Bei *B. empetrifolia* sah ich dass in einer sehr grossen Zahl von Fällen, bei *B. Darwinii* vorläufig aus Materialmangel erst in wenigen Zählungen. Die Metaphasen mit weniger als 14 Chromosomen möchte ich teilweise Verklumpung zurückführen. Ganz klar, in mehr als 150 Fällen, zählte ich dann wieder 14 Chromosomen beim Bastard. In Grösse und Form der Kernsegmente habe ich bisher keine principiellen Differenzen aufdecken können; Eltern und Bastard dürften morphologisch darin sehr ähnlich sein.

Der sehr merkwürdige Befund war beim Bastard der, dass die Reduktionsteilung genau wie bei den Eltern eine überaus regelmässige ist. Nichts, aber auch gar nichts, deutet während dieser Zeit darauf hin, dass die leisesten Störungen in der Spindelbildung oder Chromosomenverteilung sich bemerkbar machen. Spirem, Diakinese, sowie beide Reifungsspindeln erscheinen völlig regulär. Die 4 jungen Pollenkörner liegen demnach in normalem Tetradenverband und trennen sich dann genau so wie bei den Eltern. Selbst das Auskeimen des reifen Pollens ist noch zum grossen Teil gut. Freilich keimte er bei den Eltern erheblich besser. Alle Grössenklassen waren dabei beteiligt. Die Maximalbreite während des Liegens in Zuckerlösung war bei *Berberis Darwinii* ca. 60μ, bei *B. empetrifolia* ca. 55μ. Den grössten keimenden Pollen von *B. stenophylla* mass ich unter gleicher Bedingung mit 52μ Breite. Das bedeutet tatsächlich

gleiche Grösse aller drei Pollensorten. Die Keimung des Bastard-pollens war entschieden "launischer" als bei den Eltern. Manchmal keimten gegen 50% (mehr wohl niemals!), manchmal keimten nur wenige Körner. Der "Reifungsgrad" ist bei *B. stenophylla* vielleicht etwas enger oder die zum Auskeimen nötige Constellation der Aussenbedingungen weniger oft vorhanden. Die Hauptsache bleibt aber die principielle Möglichkeit für alle "Individuen" des *stenophylla*-Klons, zu einem ungewöhnlich hohen Prozentsatz gut keimenden Pollen producieren zu können. Das gleiche sehen wir bei der Entwicklung des Embryosacks. Schon 1903 war mir aufgefallen, dass die 8-Kernigkeit die Regel ist, die Makrospore somit normal keimen muss. Erneutes eingehendes Studium an neuen Präparaten bestätigte mir durchweg die alten Daten.

Trotzdem ist der Frucht- und Samenansatz im allgemeinen schlecht. In Kiel habe ich an unseren jährlich überreich blühenden Sträuchern überhaupt nie eine reife Frucht gesehen. In Heidelberg war das Gleiche der Fall; lediglich 1904 sah ich hier eine grosse Zahl reifer Früchte. Damals wusste ich noch nicht, dass das etwas seltenes war, und ich sammelte nichts. Wenn Usteri ('99) von "grosser Fruchtbarkeit" spricht, wenn er dies Factum gar gegen die Hybridnatur der *B. stenophylla* auswerten will, so bedeutet das sicherlich eine ganz seltene Ausnahme, ist aber andererseits wegen der principiellen Möglichkeit des Genotyps, in grösserem Massstabe gute Früchte hervorzubringen, sehr interessant. College Schürhoff teilte mir jüngst noch brieflich mit, dass im Berliner botanischen Garten nach Aussage des betreffenden Obergärtners auf etwa 100 gm. Beeren höchstens 3–4 Pflänzchen sich entwickelten. Das zeigt wohl genugsam, wie viele ungünstige Gencombinationen zusammentreten. Und trotzdem dann dieses, wie wir annahmen, auf "Eiweissverwandtschaft" beruhende Zusammenarbeiten der beiden Plasmen während der Reduktionsteilung und noch in den jungen Sporen! Shull hatte mündlich, wie Hurst ('25, p. 383) berichtet gemeint, *Berberis stenophylla* könne selbststeril sein. Das ist *in sensu strictiori* jedenfalls unmöglich, sonst dürften ja gar keine keimfähigen Samen entstehen. Eine Rückkreuzung mit den Eltern kommt für die Exemplare in Berlin, Heidelberg und Kiel nicht in Betracht. So konnten höchstens einzelne Sterilität verursachende Gene im Spiel sein. Hurst gab schon 1913 (s. '25) an, dass er ca. 3000 Pflänzchen der F_1 und folgenden Generation gezogen hätte. Die Variabilität war im Gegensatz zu seiner ersten Annahme ungeheuerlich gross, und "not a single plant has yet been found with all the characters of either of the original species." Die Annahme einer regulären Befruchtung der beiderlei *stenophylla*-Gameten war darnach schon sehr wahrscheinlich. Ich habe nun auch cytologisch das Problem verfolgt, und ich kann wenigstens jetzt sicher sagen, dass normale Embryo- und Endospermbildung vorhanden ist. Wenigstens spricht nichts für eine Parthenogenesis.

Wichtig wird besonders auch die Untersuchung der F_2-Individuen sein. Ein paar reife Samen erhielt ich durch die Freundlichkeit von Herrn Collegen Janse in Leiden. Zwei davon keimten aus, und einer der beiden Keimlinge kam auch zur Bildung des ersten Laubblättchens. Dann verfärbte er sich aber und starb ab. Ich konnte das Material nicht mehr cytologisch verwerten. Dagegen habe ich

durch die Freundlichkeit von Herrn Collegen Schürhoff in Berlin einige Wurzelspitzen von den in Berlin cultivierten Sträuchern der F_2-Generation, in Carnoy fixiert, erhalten. Mitosen waren nur wenige darin. Einmal zählte ich ca. 28 diploide Chromosomen; niemals war ich bisher veranlasst, eine Verdoppelung des Satzes wie bei den oben erwähnten fertilen Hybriden in Erwägung zu ziehen. Ich werde natürlich auch hier die Frage weiter verfolgen, glaube aber kaum, dass sich an dem Ergebnis viel ändern wird. Der einzige Weg, das Verhalten von *Berberis stenophylla* wirklich zu verstehen, war für uns der, dass wir weitgehende Plasmaähnlichkeit resp. Eiweissverwandtschaft annahmen. Damit möchte ich einem Zusammenarbeiten von Cytologie und Serologie das Wort reden. Es könnte z. B. eine cytologische Untersuchung der teilweise fruchtbaren Hybriden von *Berberis*, *Magnolia*, *Nymphaea*, *Nuphar*, *Aquilegia*, etc. vorgenommen und dann festgestellt werden, ob eine solche Eiweisspecifität wie bei der "abgeleiteten" Familie der Gräser (*Triticum*, *Avena*) vorhanden ist. Natürlich weiss ich z. Z. nicht, ob sich meine Vermutung bezüglich Sterilitätszusammenhanges mit systematisch-phylogenetischer Stellung wird bestätigen lassen. Aber denken wir nur an die Beziehungen zwischen Phylogenie und dem Befall verschiedener Wirtspflanzen durch Parasiten oder Gallen-erzeugende Tiere (Hering '25, Karny '26). In der Möglichkeit der Verknüpfung zweier getrennter Arbeitsgebiete sehe ich allgemein einen Weg, zu neuen Gedanken und damit zu neuen Aufgaben zu gelangen.

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AN ATTEMPT TO ANALYZE THE COMPOSITION OF NUBBIN, A COMPOUND (2n+1) CHROMOSOMAL TYPE IN DATURA (ABSTRACT)[1]

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Nubbin is a unique type in *Datura Stramonium*, with a single extra chromosome, which appeared in a culture from a flower treated by Dr. Gager with radium emanation.[2] Its breeding behavior and morphology indicated relationships with other types and enabled an analysis to be made of its chromosomal constitution.

Nubbin, in addition to itself and normal 2n offspring, throws two new types with single extra chromosomes, Pinched and Hedge, and also the two primary (2n+1) types, Buckling and Echinus. In capsule shape it strongly resembles Strawberry, the secondary of Buckling, and in pollen dimorphism (half of the grains lacking starch) it is like Mutilated, the secondary of Echinus. Its morphology will be satisfied if we consider Nubbin to have in excess the Strawberry half of the Buckling chromosome plus the Mutilated half of the Echinus chromosome. There would still have to be explained the production of Pinched and Hedge and the relation of Nubbin to Rolled through the abnormal ratios which it throws when heterozygous for "B" whites.

Pinched resembles Sugarloaf, the secondary of Rolled and throws, in addition to itself, Buckling and Rolled. Hedge resembles Polycarpic, the other secondary of Rolled, and throws Rolled and also Echinus. The evidence seems to indicate that Pinched is a (2n+1) type with the extra chromosome made up of the Sugarloaf half of the Rolled chromosome joined to the Strawberry half of the Buckling chromosome, while the extra chromosome in Hedge is made up of the Polycarpic half of the Rolled chromosome joined to the Mutilated half of the Echinus chromosome. The formula for Pinched would be $2n+\frac{1}{2}$ Sg $\frac{1}{2}$ St while that for Hedge would be $2n+\frac{1}{2}$ Py $\frac{1}{2}$ Mt.

Nubbin is believed to have both the compound chromosomes of Pinched and Hedge but to possess only one intact Rolled chromosome. The formula for Nubbin, therefore, would be $2n-1$ Rl$+\frac{1}{2}$ Sg $\frac{1}{2}$ St$+\frac{1}{2}$ Py $\frac{1}{2}$ Mt. The lack

[1] Presented before the International Congress of Plant Sciences, Section of Genetics, New York, Aug. 18, 1926. A full discussion with tables and illustrations will be given in a paper awarded the A. Cressy Morrison Prize No. II by the New York Academy of Sciences and now in press in the Memoirs of the Academy. A summary with chromosomal diagrams is in press in Proceedings, National Academy of Sciences.

[2] Gager, C. Stuart, and A. F. Blakeslee. Chromosome and gene mutations in Datura following exposure to radium rays. Proceedings National Academy of Sciences, February, 1927.

of an intact Rolled chromosome is made good by the presence of its two halves—Sugarloaf and Polycarpic—in the two compound chromosomes.

The permanent attachment of parts of non-homologous chromosomes which seems to have taken place in the formation of Nubbin may be a similar phenomenon to that responsible for the "B" whites[3] and for the lines in nature which produce hybrids with 50 per cent aborted pollen.[4]

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[3] Belling, John, and A. F. Blakeslee, 1924. On the attachment of non-homologous chromosomes at the reduction division in certain 25-chromosome Daturas. Proc. Nat. Acad. Sci. **12:** 7–11.

[4] Blakeslee, A. F., and J. L. Cartledge. Sterility of pollen in Datura. In press, Proceedings International Conference on Flower and Fruit Sterility.

A CASE OF LINKAGE OF A HIGHER ORDER[1]

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Even in 1914 my attention was drawn to the phenomenon of linkage in wheat. At that time I expressed the supposition that the factor producing the long ears of *Triticum Spelta* is coupled with that producing the shapes of glumes and spikelets characteristic of this species of wheat.

The shapes of the glumes of *T. Spelta*, I considered, at that time, to be a single Mendelian character. *T. Spelta* crossed with *T. vulgare* gives in F_1 generation intermediate individuals and in F_2 appears a segregation into three types, namely: *T. Spelta*, intermediates, and *T. vulgare* in the proportion of 1:2:1.

My observations were confirmed in 1923 by B. Kajanus. This author supposes, however, that the shapes of glumes and spikelets and with them also the characteristic length of ears of *T. Spelta* are caused by the presence of one Mendelian factor only. My later investigations have shown that the shapes and sizes of glumes and spikelets do not constitute, by any means, a simple Mendelian character. In 1918 I suggested that the shapes and sizes of the glumes of *T. vulgare*, *T. dicoccum*, and *T. Spelta* are determined by numerous Mendelian factors. This conclusion was based upon the results of crossing *T. vulgare* with *T. dicoccum*. In the F_2 from this cross numerous new types have appeared, among them *T. Spelta* and *T. durum*. In 1912 I suggested that in the cross *T. vulgare*×*T. Spelta* the factors producing the shapes and sizes of glumes and spikelets are linked in these two species. At the same time I believed that the phenomenon of crossing-over occurs in the F_1 of the cross *T. vulgare*×*T. dicoccum*, and that the aggregate of numerous factors, mentioned above, breaks up in this cross. It seemed probable to me that by means of Morgan's theory of linkage and crossing-over these phenomena could be explained. However, when I tried to bring all the observed phenomena into line with Morgan's theory, I noticed various difficulties.

Some of those difficulties were: (1) I could not explain why the supposed phenomenon of crossing-over might be expected to occur in the cross *T. vulgare*× *T. dicoccum* and yet did not appear in the crosses *T. vulgare*×*T. Spelta* or *T. dicoccum*×*T. polonicum*. (2) I could not explain why the individuals of one of the parental types are more numerous in the crosses *T. polonicum*×*T. vulgare*, *T. dicoccum*×*T. vulgare*, *T. polonicum*×*T. Spelta*, so that the individuals of the F_2 reproducing the types *T. polonicum* and *T. dicoccum* were more numerous than those representing the types *T. vulgare* and *T. Spelta*. (3) I could not

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[1] Presented before the International Congress of Plant Sciences, Section of Genetics, Ithaca. New York, Aug. 18, 1926.

explain why the individuals reproducing the parental types in the crosses between tetraploid and hexaploid species appeared in such small numbers as compared with such other types as appeared in the F_2.

My experiments, taken together with the cytological observations of Sakamura and Kihara, suggested that we have to deal here with a linkage of a higher order due possibly to an association between the chromosomes.

I found in the recent paper of Bateson an opinion analogous to my own. For, examining the results of Engledow's investigations on the cross *T. polonicum*×*T. durum*, Bateson believes that the "association of characters may be due to an association of particular chromosomes."

It was established by Sakamura that the four species of wheat, namely: *T. dicoccum*, *T. polonicum*, *T. durum*, and *T. turgidum* are tetraploid, each possessing 28 chromosomes, whilst the species *T. vulgare* and *T. Spelta* are hexaploid and contain 42 chromosomes. The phenomenon of linkage between factors determining shapes and sizes of glumes obtains so long as we cross the species containing equal numbers of chromosomes. But on crossing a tetraploid species with a hexaploid one the linkage ceases to exist. This fact suggests the idea that linkage phenomena are dependent upon intercourse between chromosomes. It was stated by Sax and Kihara that in the crosses *T. durum*×*T. vulgare* and *T. polonicum*×*T. Spelta* the F_2 plants possessing 28 chromosomes always reproduce the morphological type of the tetraploid parent. The F_2 plants possessing 42 chromosomes, on the contrary, always reproduce the morphological type of the hexaploid parental species. My own observations have shown that the species *T. Spelta*, obtained in the F_2 on crossing *T. dicoccum* with *T. vulgare*, possesses 42 chromosomes. *T. Spelta*, appearing in the F_2 from the cross *T. polonicum*×*T. vulgare*, contains the same number of chromosomes, that is, 42. The species *T. dicoccum*, appearing in the F_2 from the cross *T. polonicum*×*T. vulgare*, possesses 28 chromosomes. *T. durum* received by me from a cross *T. polonicum*×*T. vulgare* possesses also 28 chromosomes. All these facts prove that it is the number of the chromosomes that decides the shapes and sizes of glumes and spikelets. The question arises whether the quality of chromosomes plays a part in the differentiation of species of wheat. Doubtless the constitution of chromosomes of each tetraploid species is of a different quality, and this also holds good for the hexaploid species. Sax, dealing with hybrids *T. vulgare*×*T. durum*, assumes that the 7 additional chromosomes of *T. vulgare* determine the distinguishing characters of the common wheats, and that these characters are due either to a reduplication of hereditary factors or to specific factors in these chromosomes. The second of these alternatives seems more probable to me. We know several tetraploid species differing from each other, from the morphological point of view, and it would be impossible to assume that they are all uniform in respect to the genetic constitution of the chromosomes, bearing the factors for shapes and sizes of glumes and spikelets.

But the most important conclusion to be drawn from these observations is that the number of the chromosomes, as well as their constitution, plays a significant part in the production of specific differences.

Factors producing specific characters are located in a smaller number of chromosomes in the tetraploid species than in the hexaploid ones. If we assume that the factors determining shapes and sizes of glumes and spikelets in the tetraploid species are located in a minimum of two non-homologous chromosomes linked one with another, we must admit that this minimum is represented by three non-homologous chromosomes in the hexaploid species. These chromosomes are associated, and they pass, therefore, together to the one pole of the dividing cell.

In a pentaploid hybrid the linkage between the chromosomes breaks, and for this reason new combinations of chromosomes may originate. We know that in these hybrids 14 gemini and 7 solitary chromosomes may be observed. These solitary chromosomes often remain outside of the newly formed nucleus. The intracellular equilibrium becomes unstable, and the breaking of the hypothetical association between chromosomes in these circumstances seems to me very probable.

If we admit that the characters of glumes and spikelets are determined by a great number of factors located in several associated chromosomes, the three difficulties I have mentioned above are easy to overcome.

It becomes clear why the linkage exists in the cross *T. vulgare*×*T. Spelta* and ceases to exist in the crosses *T. vulgare*×*T. dicoccum* and *T. vulgare*×*T. polonicum*. It is because in both the last named crosses the two sets of chromosomes of different number unite.

It then becomes clear why the individuals of the F_2 generation reproducing the morphological type of the tetraploid parent are more numerous than the individuals reproducing the morphological type of the hexaploid parent. It is because the chances of the appearance of a combination of a smaller number of definite chromosomes are greater than the appearance of a combination of a larger number of definite chromosomes.

One or more of the solitary chromosomes in the F_1 hybrids often remain out of the newly formed nucleus. The elimination of one of the univalent chromosomes is the most frequent phenomenon. This may be one of the causes of the numerical preponderance of the tetraploid individuals in the F_2 generation.

It becomes clear why the F_2 individuals, reproducing the parental types, are relatively less numerous than the other types appearing in this generation. It is because in the pentaploid hybrids the aggregate of linked chromosomes breaks, and in F_2 the parental combinations of chromosomes, following the laws of chance, must be less numerous than the sum of other combinations.

The phenomenon of the occurrence in the F_2 generation of various species beside the parental types proves that the parental types contain the elements determining those species. If we assume that these elements are chromosomes, then the various combinations of them could produce all the species in question. We have admitted that in the tetraploid species 2 chromosomes, and in the hexaploid 3 chromosomes, are linked. Since in these linked chromosomes are located factors of specific characters, it is possible that a hexaploid species might possess 2 chromosomes in common with a tetraploid one. Con-

sequently, in certain crosses we should obtain less complicated segregations than in others.

In my experiments the most complicated segregations have appeared in the crosses *T. polonicum*×*T. vulgare* and the least complicated in the crosses *T. dicoccum*×*T. Spelta*.

In the F_2 generation from the cross *T. polonicum*×*T. vulgare* I have obtained the species *T. polonicum*, *T. dicoccum*, *T. durum*. *T. Spelta*, and *T. vulgare*. In the F_2 from the cross *T. dicoccum*×*T. vulgare* I have observed the species *T. dicoccum*, *T. durum*, *T. Spelta*, *and T. vulgare*. In the F_2 from the cross *T. polonicum*×*T. Spelta* I have observed the species *T. polonicum*, *T. dicoccum*, and *T. Spelta*. In the F_2 from the cross *T. dicoccum*×*T. Spelta* I have found only the species *T. dicoccum* and *T. Spelta*.

From the results of my experiments and of those of other authors I try to find the degrees of relationship between particular species of wheat.

The cross *T. polonicum*×*T. vulgare* has given, in my experiments, the greatest heterogeneity as regards the F_2 generation, and I have observed the smallest in the cross *T. dicoccum*×*T. Spelta*. This difference in the degree of heterogeneity may indicate that the species *T. dicoccum* and *T. Spelta* have certain chromosomes in common.

Let us suppose that *T. polonicum* contains two connected chromosomes *a* and *b*; *T. dicoccum*, two connected chromosomes *a* and *c*; and *T. durum*, *e* and *d*. Since *T. vulgare* crossed with *T. durum* gives a relatively uniform F_2 generation, we may assume that these two species have the chromosomes *c* and *d* in common and that *T. vulgare* contains in addition the *e* chromosome. *T. Spelta* crossed with *T. dicoccum* gives also a small heterogeneity in the F_2 generation. We may assume, therefore, that *T. Spelta* in common with *T. dicoccum* possesses two chromosomes *a* and *c*. The variation of the F_2 generation from the crosses *T. polonicum*×*T. vulgare* is greater than in the crosses *T. dicoccum*×*T. vulgare*. For that reason we may assume that if *T. polonicum* has not one single chromosome in common with *T. vulgare* there must exist at least one common chromosome (for instance *c*) in both *T. dicoccum* and *T. vulgare*.

In considering the relationship of wheats I have taken into account chiefly the sizes and shapes of glumes and spikelets.

It is obvious that beyond the complexes of associated chromosomes in each species, factors influencing shapes of glumes may exist, and a complete reconstruction of parental species in the posterity of pentaploid hybrids may therefore in such circumstances be difficult to obtain. If the factors in question really exist they are probably more numerous in hexaploid species because these species contain a greater number of chromosomes. The existence of these factors still more diminishes the chances of the appearance of the hexaploid parental types as compared with that of the tetraploid ones. The segregation phenomena are in reality more complicated than they would be if all the factors determining the specific differences were located only in two or three associated chromosomes.

SPECIES HYBRIDIZATIONS AMONG OLD AND NEW SPECIES OF SHEPHERD'S PURSE[1]

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TABLE OF CONTENTS

	PAGE
Introduction	837
Methods	839
The species of Bursa	840
Bursa bursa-pastoris (Linnaeus) Britton	842
Bursa bursa-pastoris apetala (Opiz)	845
Bursa Heegeri (Solms-Laubach)	846
Bursa occidentalis n. sp	847
Bursa occidentalis madeirae n. subsp	849
Bursa orientalis n. sp	849
Bursa djurdjurae n. sp	851
Bursa penarthae n. sp	851
Bursa grandiflora (Boissier)	852
Bursa rubella (Reuter)	854
Bursa Viguieri (Blaringhem)	855
Bursa tuscaloosae n. sp	856
Other species	858
Interspecific hybridizations	859
Crosses of *Bursa Heegeri* with other species of the first group	860
Other crosses between species of the first group	865
Species of the second group crossed with *Bursa Heegeri* and *B. bursa-pastoris*	865
Crosses of other first-group species with species of the second group	869
Crosses between species within the second group	871
Bursa grandiflora×*B. Viguieri*	871
Bursa rubella×*B. Viguieri*	874
Bursa grandiflora×*B. rubella*	875
Bursa rubella×*B. tuscaloosae*	876
Bursa grandiflora×*B. tuscaloosae*	876
Other combinations within the second group	877
The significance of the two groups of species	878
Discussion	880
Summary	885
Literature cited	887

Introduction

The present paper is a progress report on a research program in which the aim has been to articulate genetical investigations with three other fields of biological endeavor: taxonomy, phytogeography, and cytology. This program

[1] Presented before the International Congress of Plant Sciences, Section of Genetics, Ithaca, New York, Aug. 18, 1926.

had its inception in an attempt to carry Mendelian analysis beyond the field of domesticated plants and animals into the realm of wild nature where relationships have been unmodified by artificial breeding and selection by human agency. After the initial demonstration (Shull 1907, 1909, 1910) that the Mendelian principles are in full operation in *Bursa*, the program has had as its leading motif the attempt to trace the geographical distribution of the duplicate factors for the characteristic triangular form of the capsules in the common shepherd's purse (*Bursa bursa-pastoris*) as contrasted with the uninflated, top-shaped capsules of *Bursa Heegeri*. In addition to this central theme, numerous other problems have presented themselves as materials of diverse character have been accumulated and extensively utilized in cross-breeding experiments. Important progress has been made toward the solution of many of these problems which will supply subject matter for future reports.

The first pedigree-cultures in this series of investigations were grown at the Station for Experimental Evolution, of the Carnegie Institutution of Washington, in 1905, but after two years they were omitted from the cultures for three years to permit the expansion of studies on several other plant species. In 1910 the *Bursa* cultures were resumed and have been continued extensively every year since, except that in the winter of 1913-14 only a few families were grown, as I spent that winter in Berlin, and grew only 17 cultures of *Bursa* and 3 of *Lychnis* (*Melandrium*) for which Doctor Erwin Baur kindly provided space and facilities on his experimental grounds at Friedrichshafen. The cultures were continued at the Station for Experimental Evolution until the autumn of 1915 when they were transferred to Princeton University, where they have been maintained ever since.

Some conception of the magnitude of this study may be gained from the fact that nearly 4000 pedigreed families have been grown to date, including a total of considerably over half a million individuals. The original collections which form the various starting points for these cultures have been made by a very large number of correspondents whom it will not be possible to enumerate at this time, though their assistance has been greatly appreciated and I can not too warmly express my gratitude to all who have so generously aided in securing this valuable material (and, by anticipation, to those in various parts of the world who will in future aid in the continuation of this investigation by sending still further collections). These original collections already exceed 300 in number and represent about 100 localities in Wales, Holland, Sweden, France, Germany, Czechoslovakia, Switzerland, Italy, Greece, Madeira Islands, Algeria, Natal, India, Ceylon, China, Japan, Korea, Formosa, Siberia, Australia, Tasmania, Peru, Chile, Argentina, Uruguay, Sandwich Islands, Vancouver, and nineteen of the United States of America.

It will be obvious that in attempting to present a brief comprehensive view of the results of so extensive a series of genetical studies, general summaries and conclusions must be given with a minimum of supporting data, the details being reserved for papers dealing with the numerous special problems which have presented themselves for solution.

Methods

In the beginning of these cultures the attempt was made to grow several generations a year, but it was soon found that the plants do not develop well during the summer. In warm weather they run up tiny, weak flowering-stems, precociously, from juvenile rosettes, the length of period of diurnal illumination probably having something to do with this unsatisfactory behavior. It soon became the settled policy, therefore, to start the *Bursa* cultures in the fall, and replace them toward spring by the expanding cultures of other plants,—*Lychnis*, *Oenothera*, etc.,—which have made up the summer cultures in the experimental garden. All cultures of *Bursa* have been started in square earthen seed pans or wooden boxes of sterilized soil in the greenhouse and set, at the

Fig. 1. Pedigree-cultures of *Bursa* in the rosette stage at Princeton University in 1915–1916.

appropriate stages, singly, into 7 cm. pots in which they have completed their development. (Figs. 1 and 2.) On two occasions a portion of the plants were later reset from the pots into the open ground in the experimental garden, and quite generally coldframes have been utilized to house such portions of the cultures as overflowed the available greenhouse space. Except for certain problems concerned with leaf-lobing and with pollen-sterility the coldframe-grown plants have proved satisfactory, but resetting to the open ground has not given good results, many of the plants having been lost when this method was resorted to.

The habit of *Bursa* makes it a favorable object for genetical study, since the nearly leafless stems may be kept enclosed in paper bags during their entire fruiting period without producing noticeable injury to the plants. The only unfavorable feature of these plants for genetical investigations is the small size of the flowers, which makes the process of castration somewhat tedious. Slight protandry makes necessary the castration of the flowers about 24 hours

before the stage of anthesis is reached, and occasionally some accidental selfing takes place because of the early bursting of the anthers in contact with the stigmatic surface. Such accidents have been rare, however, and have been practically always readily recognized on inspection of the progenies. The castrations are simply performed by cutting away with a needle-pointed forceps all of the floral envelopes and the six stamens, leaving only the naked pistil, to the stigma of which the appropriate pollen is applied the following day. The application of the pollen is made by removing with the forceps whole flowers from the chosen male parent, which also has been previously enclosed in a paper bag, and touching the open anthers directly to the stigmas of the castrated flowers. As the full

Fig. 2. Pedigree-cultures of *Bursa* in full bloom at Princeton University, March 6, 1922. Photograph by W. P. Agnew.

capsule usually contains 10 to 20 seeds, a series of ten or more castrations is usually made in preparation for each cross. After the desired number of castrations has been completed, the remaining buds of the raceme are removed, and in about four weeks after the last pollination the mature seeds are ready for collection. In all species except *B. grandiflora* and a pollen-sterile segregate of *B. bursa-pastoris* self-fertilization takes place automatically, and controlled selfing is provided for by simply enclosing the inflorescence in a paper bag and leaving it in place until the plant has reached full maturity. Even when not enclosed, self-fertilization takes place almost exclusively, the percentage of crossing in greenhouse-grown cultures being practically zero.

The Species of Bursa

In order that one may intelligibly discuss any extensive series of investigations on living organisms, it is necessary to adopt a terminology which will

allow groups of individuals to be mentioned by names which are not necessarily descriptive of all their characteristics, but which will imply all the known differentiating features, as well as any additional ones which further investigation may bring to light. For forms growing in nature the binomial nomenclature adopted by Linnaeus has commended itself for universal use for all groups which can be successfully delimited in a descriptive sense without involving too difficult or too trivial single differentiating features. As I have pointed out more than once (Shull 1923, 1929), specific distinctions can not be practicably based upon every genetical difference that exists, and consequently most species are composites of more than one biotype. While the biotypes are, therefore, strictly natural entities, species are as a rule only quasi-natural. They rest on the judgment of the taxonomist as to what distinctions are practicable and useful in understanding the composition and relationships of the particular group of organisms in question.

In the taxonomic history of the genus *Bursa* there have been manifested the same differences of judgment that have characterized the treatment of many other genera. Most of the manuals recognize only the species *Bursa bursa-pastoris*, sometimes with the mention of subspecies *apetala*, *rubella*, *integrifolia*, etc., while certain students have attempted to name all forms whose progenies can be distinguished from one another when grown side by side in the garden. Alexis Jordan (1864) was the first to adopt essentially this latter viewpoint and he described five forms of *Bursa* as new (elementary) species. De Borbás (1902) recognized and described about 30 constant forms growing in Hungary. More recently E. Almquist (1907, 1921) has carried the same method to a much greater extent, and has proposed nearly 200 "elementary species" which he usually speaks of as "species" but to which he has given trinomial names. He treats even such distinct species as *B. rubella* and *B. grandiflora* as subspecies (elementary species) of *B. bursa-pastoris*. While we note with sympathetic appreciation such efforts to make taxonomic groups agree with biotypes, thus making them *natural*, it is easy to demonstrate that such an aim is impossible of complete attainment, and that such an attempt to name all wild biotypes defeats the purpose of taxonomic classification, by making the names too numerous and their application too uncertain.

I have found it convenient to recognize in the material which has been involved in these cultures the following ten species and two subspecies, which fall into two natural groups with respect to their genetical behavior:

First Group (the *bursa-pastoris* group)

a. *Bursa bursa-pastoris* (Linnaeus) Britton
b. *Bursa bursa-pastoris apetala* (Opiz)
c. *Bursa Heegeri* (Solms-Laubach)
d. *Bursa occidentalis* n. sp.
e. *Bursa occidentalis madeirae* n. subsp.
f. *Bursa orientalis* n. sp.
g. *Bursa djurdjurae* n. sp.
h. *Bursa penarthae* n. sp.

Second Group (the *rubella* group)

i. *Bursa grandiflora* (Boissier)

j. *Bursa rubella* (Reuter)

k. *Bursa Viguieri* (Blaringhem)

l. *Bursa tuscaloosae* n. sp.

These ten species and two subspecies will be briefly characterized before taking up the results of the various interspecific hybridizations. From a certain point of view it would be more logical to consider the second group of species first, since this is undoubtedly *the more ancient group*, but the wide distribution of *B. bursa-pastoris*, and its place of almost exclusive prominence in the manuals, seem to necessitate the making of this most familiar species the principal basis of comparison.

BURSA BURSA-PASTORIS (LINNAEUS) BRITTON

This species is usually characterized by having capsules which are longer than wide, with more or less convex or sometimes slightly sigmoid or nearly rectilinear lateral margins, nearly truncate at apex, the apical sinus being usually shallow (but sometimes deep and narrow), and the angles not notably divergent. The foliage is rather dark green, herbaceous, variously lobed or sometimes nearly unlobed. The inflorescence usually has a more or less prominently developed series of sterile flowers on the proximal portion of the terminal raceme, and these are also occasionally seen on lateral racemes, but usually the lateral racemes have no sterile flowers at base even when such sterile flowers are abundant on the central raceme (Figs. 4 and 22).

Many distinct biotypes are included in this species. Four fundamental leaf-lobe types have been found widely distributed and have been given the names *heteris*, *rhomboidea*, *tenuis* and *simplex*, as described in several of my previous papers (see Fig. 3). These are produced by the interplay of two pairs of Mendelian factors, *Aa* and *Bb*, giving the four combinations *AB*, *aB*, *Ab* and *ab*. The *B* factor is occasionally duplicated, yielding approximately 15:1 ratios in F_2 progenies. As will be seen below in the section on crosses with *B. Heegeri*, the triangular form of capsule is determined by duplicate Mendelian factors, *C* and *D*, giving likewise 15:1 ratios in F_2 from crosses with *B. Heegeri*. Only two plants have yet been found in which the capsule factors were not duplicated. One of these was reported by Dahlgren (1915) from Upsala, Sweden; the other I received from Landau, Germany, where *B. Heegeri* was originally discovered. For the latter case the explanation is reasonable, that the plant which showed no duplication of the capsule factors was a monomeric derivative from a previous natural cross between *B. bursa-pastoris* and *B. Heegeri*. Among other factors which have been discovered in *B. bursa-pastoris*, a recessive "*coriacea*" factor, *k*, produces coriaceous texture of the leaves with much-reduced lobing (Fig. 5). Its allelic dominant factor, *K*, which produces the typical herbaceous foliage, is likewise usually duplicated in this species. The *coriacea* factor, *k*, is closely linked with a factor for dwarf stems (10 to 30 cm. tall) (see Fig. 4). The normal allele of another recessive factor, s_p, which produces pollen-sterility, is also

sometimes duplicated (Shull 1927), but this last-mentioned case is not yet adequately studied.

Bursa bursa-pastoris appears to be generally distributed in the temperate regions of Europe, Asia, Australia, Africa, Canada, and all but the southwestern portions of the United States. Over 200 of my original collections have been

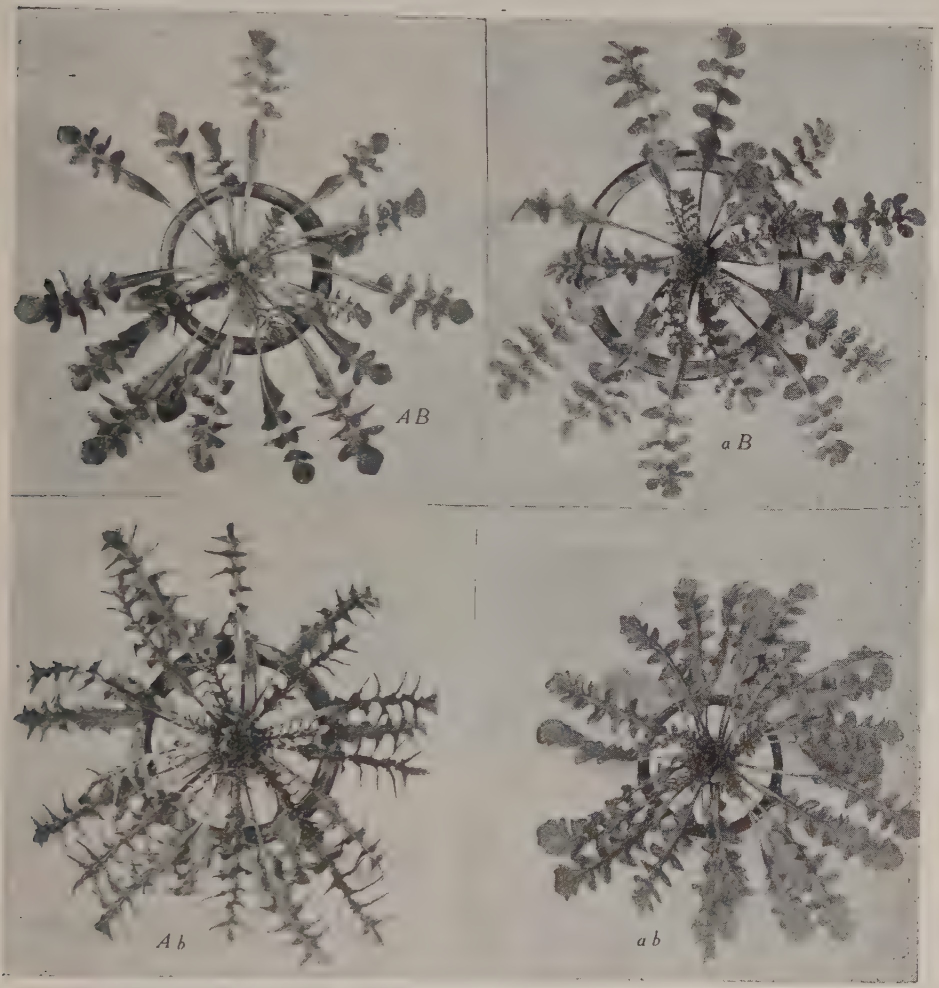

Fig. 3. The four rosette-types commonly present in the various species and biotypes of the genus Bursa. In *Bursa penarthae*, *B. Viguieri*, and certain biotypes of *B. bursa-pastoris* these rosette types have been found modified by partial inhibitors.

clearly referable to this species. The only temperate region of considerable magnitude from which this species has not yet been received is the area from Tucson, Arizona, westward along the southwestern border of the United States, and up and down the Pacific Coast from Santa Rosa, California, southward, and it has not yet come to me from any South American locality, though I have

Fig. 4. *Bursa bursa-pastoris heteris* and dwarf segregate, *B.b.p. coriacea*. Note the sterile flowers at base of central axis.

Fig. 5. Climax leaves of typical *rhomboidea* rosettes and of *rhomboidea coriacea* (at right), showing the effect of the recessive *coriacea* factor *k*.

had over 50 collections of *Bursa* seed from four of the South American countries, —Peru, Chile, Argentina, and Uruguay.

BURSA BURSA-PASTORIS APETALA (OPIZ)

This striking subspecies has the four petals replaced by four staminodia which have nearly the form of the true stamens, but with indehiscent anthers which are sometimes slightly petaloid at the top (Fig. 6). This form was first described in 1821 by Opiz who discovered it at Prag, Czechoslovakia, and studied it in garden culture. It has since been widely distributed in botanical gardens. My material

Fig. 6. Flowering corymbs of *Bursa bursa-pastoris* and of its subspecies, *B. b.p. apetala.*

was received in 1914 from the Bremen Botanical Garden, through the kindness of the Director, Doctor G. Bitter. This subspecies is not to be confused with the many cases of partial apetaly which have been observed and reported from time to time by various authors. I have also, on several occasions, found these sub-apetalous variations in cultures unrelated to *apetala*, but they have always been only partially apetalous, the development of petals becoming more accentuated as the racemes advanced. *B. bursa-pastoris apetala* (Opiz) is about equally apetalous in both young and older racemes. The capsules are fairly large, of typical *bursa-pastoris* form, and the rosettes are well-rounded *rhomboidea*, smooth and dark green. The apetalous character has behaved in my cultures as a typical monohybrid Mendelian (partially) dominant trait, as Dahlgren (1919) has also reported it to be.

BURSA HEEGERI (SOLMS-LAUBACH)

This species is characterized by its round, top-shaped, uninflated capsules (Figs. 7 and 8), the valves lacking or nearly lacking the "parchment layer" which gives to other species the rigidity of the capsule walls (Shull 1914). The valves commonly do not fall off by a definite articulation, the walls being so thin and weak that the seeds make their exit through the torn or disintegrated valves. The seeds, growing in contact with each other, become irregularly angular instead of prolate-spheroidal as in the other species of *Bursa*. No conspicuous differences

Fig. 7. Herbarium specimen of *Bursa Heegeri* and a fruiting raceme segregated from crosses with other species of the *bursa-pastoris* group.

occur in other characters to differentiate this species from *B. bursa-pastoris*, though *B. Heegeri* seems to be a little slower in development and also to show a slightly more conspicuous leafiness of the stems.

Bursa Heegeri has been found only once in nature in a position where it could be reasonably assumed that it had not been derived from a nearby garden culture. So far as is known, the original *B. Heegeri*, discovered by Professor Heeger on the public square in Landau, Germany, in 1897 and 1898, had rosettes of the *heteris* type (Fig. 7). At any rate my material of this species, which was secured

by Dr. D. T. MacDougal from the botanical garden of the University of Strasbourg in 1904 had *heteris* rosettes, and Almquist's figure (1907, p. 17) indicates that his material of this species had the same form of rosette.

Bursa Heegeri has given rise, repeatedly, though quite infrequently, to a mutant form which I have been calling *Bursa Solmsiana*, but which is probably identical with the f. *atavica* Wittrock MS mentioned by Almquist (1926, p. 58). This stands intermediate between *B. Heegeri* and *B. bursa-pastoris*, usually having more or less inflated capsules with deciduous valves (Fig. 8). One of the most remarkable peculiarities of this mutant type is its wide range of variation, extending almost from *B. Heegeri* on the one hand almost to *B. bursa-pastoris*

Fig. 8. Capsules of *Bursa Heegeri* (above) and of a mutant form, *B. Solmsiana* which occasionally appears in the pure cultures and segregating hybrids of *B. Heegeri*.

on the other. Very commonly a single raceme shows *Heegeri* capsules proximally but displays more and more of the *Solmsiana* character in the more distal portions of the raceme. It was very interesting to me to note in 1914 that the culture labeled "*Capsella Heegeri*" in the Botanical Garden at Steglitz-Berlin contained at that time only *B. Solmsiana*. It seems best to limit the name *Heegeri* to the round-capsuled type, except, of course, when the "atavistic" variation is produced by disease, as it sometimes is, in which case it is not hereditary.

BURSA OCCIDENTALIS N. SP.

This species has light-green, more tender foliage than *B. bursa-pastoris*, very frequently more or less conspicuously decorated with brownish anthocyan pigment on the large rounded secondary lobes ("B lobes" or "lunulae") of the leaves. The *heteris* lobing of the leaves is often modified by the partial or complete phenotypic suppression of the elongated "primary" lobes ("tenulae"), or even by the suppression of all lobing. When tenulae or "primary lobes" are well developed they frequencly show a tendency to curve strongly apicad (Fig. 9) in contrast with the corresponding lobes of *B. bursa-pastoris* which are usually

straight and spreading more or less directly laterad or only moderately apicad. The capsules are of the same size as in *B. bursa-pastoris*, but usually have slightly concave sides, and the apical sinus is relatively broad and deep, giving the angles a more spur-like aspect than in *B. bursa-pastoris* (Figs. 19 and 21). The plants are usually much more precocious in flowering than in *B. bursa-pastoris* and the occurrence of sterile flowers at the base of the flowering spike is relatively much less frequent (Fig. 22).

Seeds of *Bursa occidentalis* were first received from Doctor D. T. MacDougal in the spring of 1906, having been collected by him at Tucson, Arizona. Repeated collections made at Tucson by several different collectors in more recent years have always given the same type. Over 60 original cultures of this species have

Fig. 9. Leaves and capsules of *Bursa occidentalis* (at left) and of its subspecies, *B. occ. madeirae.*

been grown from seeds collected at Berkeley, Redlands, Riverside, and Carmel, California; Lanai, Hawaii; Chignata and La Paz, Peru; Limavida, Valparaiso, and Santiago, Chile; Angol and Tucumán, Argentina; Montevideo, Salto Department and Cerro Largo, Uruguay. One lot of seeds collected by Doctor Tine Tammes in 1914 at Groningen, Holland, seemed to belong to this species, but as the genetical behavior of *B. occidentalis* was not then so well understood as later, I feel some hesitancy in asserting now that the Groningen plants,—so remote from the rest of the range of *B. occidentalis*,—were in fact identical with those from Arizona, which was the only American station at that time known. Eight other lots of seeds received at the same time from Doctor

Tammes yielded only *B. bursa-pastoris*. Two of Almquist's elementary species, *Capsella b.p. concava* (Almquist 1907, p. 12) from Santa Rosa, California, and *C. b.p. patagonica* (Almquist 1921, p. 52) from southern Argentina, are included within this species.

It is interesting to note how closely the range of this species as indicated by the list of localities enumerated above agrees with the region occupied by the Spanish settlers in America. It seems probable that there is a causal relation between these two distributions.

BURSA OCCIDENTALIS MADEIRAE N. SUBSP.

This subspecies is closely similar to *B. occidentalis*, but differs from it in having the *rhomboidea* form of rosette leaves, without the brownish-purple pigmentation usually seen in *B. occidentalis* (Fig. 9). Like typical *B. occidentalis* the subspecies *madeirae* is precocious in development and usually shows few or no proximal flowers. Seeds of this subspecies were first sent to me in the spring of 1921 from the Madeira Islands by Professor T. D. A. Cockerell, and 15 out of 16 lots of seeds collected for me by G. L. Fawcett in 1925 at Tucumán, Argentina, were of the subspecies *madeirae*, the one remaining collection being typical *B. occidentalis*. The occurrence of this subspecies at Funchal, Madeira Islands, suggests a connection between Spain and the regions occupied by the Spanish in America. I have had no seeds yet from Spain, but it would not be very surprising if *B. occidentalis madeirae*, and perhaps typical *B. occidentalis* as well, should be found in the Spanish peninsula or Europe. In fact, Almquist (1907) reports having received his elementary species *concava* from the Botanical Garden of Coimbra, Portugal, and under *patagonica* (Almquist 1921, p. 52) he states that he has received similar forms from Coimbra, Portugal; Catania, Sicily; Cannes, Strasbourg and Nantes, France. These two elementary species of Almquist are undoubtedly included in my *B. occidentalis*, but there is still a question as to whether these European collections were truly in agreement with the South American material, as no genetical tests were made by Almquist other than to grow them in open garden beds.

BURSA ORIENTALIS N. SP.

Bursa orientalis is most notably characterized by capsules which are as broad as, or broader than, long, usually with well-curved convex sides (Fig. 10). The rosettes are dark green, usually relatively small, the lobing rather sharp and definite. All four of the major rosette types,—*heteris*, *rhomboidea*, *tenuis* and *simplex*,—are exhibited. The stems are mostly rather dwarfish and relatively slow in developing.

My first culture of this species was grown from seeds sent me in 1914, from Peking, China, by T. Z. Chang. In 1915 it was received from Peking, China; Singen, Korea; and Osaka and Fukuoka, Japan, through the kindness of the Director of the Agricultural College at Sapporo, Japan; and in 1923 it was collected for me by Professor T. D. A. Cockerell at Vladivostok, Siberia. It is clear

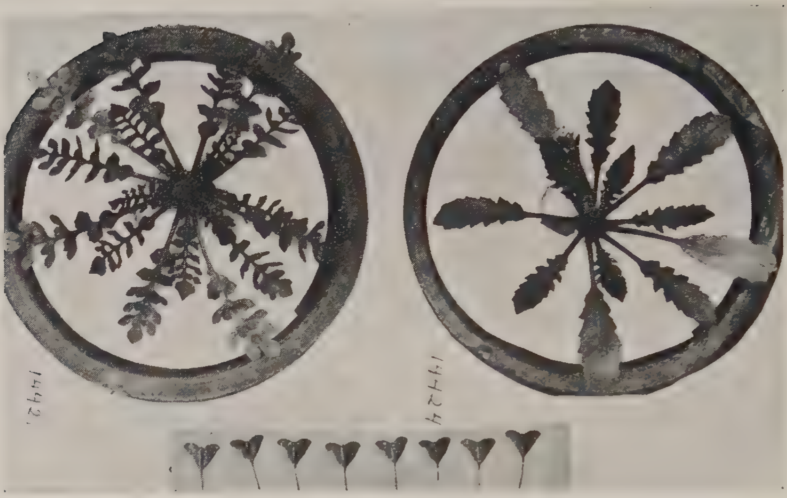

Fig. 10. Rosettes and capsules of *Bursa orientalis.* The left-hand rosette is *heteris*, the right-hand one *simplex*. The capsules were taken from eight different plants.

Fig. 11. Rosette and capsules of *Bursa djurdjurae*. Rosette photographed by W. P. Agnew.

that it has a wide distribution in Eastern Asia, where it is probably associated generally with *B. bursa-pastoris*.

BURSA DJURDJURAE N. SP.

This strikingly unique species has broad shining foliage of the *simplex* type, but with shallow sinuses between the lobes (Fig. 11). The stems are vigorous and usually rise vertically to a height of about 2 dm. then bend over to a nearly horizontal position (Fig. 12). The flowers are large, only slightly smaller than in *B. grandiflora*, self-fertilizing; the capsules are exceptionally large and not flat, but usually with a sigmoid flexure of the valves (Fig. 11) not seen in any form of *B. bursa-pastoris* which has come to my attention.

Fig. 12. Habit of *Bursa djurdjurae* showing the erect proximal portion of the stem and procumbent distal portion. Photograph by W. P. Agnew.

Bursa djurdjurae was collected for me in the spring of 1915 at Azeron-u-Tirouda in the Djurdjura region of eastern Algeria by Doctor Réné Maire of the University of Algiers, and has not yet been found elsewhere.

BURSA PENARTHAE N. SP.

Bursa penarthae is characterized by proximally tapering capsules, and rosette-leaves of partially inhibited *rhomboidea* type (Fig. 13). Two plants of this species were sent to me in 1913 by Professor A. H. Trow, who collected them at Penarth, Wales. Several other collections made at Penarth and at Cardiff, Wales, were *B. bursa-pastoris*. For several years *B. penarthae* was grown under the

temporary designation "new-form." It has not been continued in my cultures in recent years, but one of its unique genotypic factors, a recessive inhibitor of the deep lobing produced by the *rhomboidea* factor, *B*, is being maintained and utilized in various crosses.

Fig. 13. Type-specimen of *Bursa penarthae*. This specimen was collected by A. H. Trow, at Penarth, Wales, September 23, 1913.

BURSA GRANDIFLORA (BOISSIER)

Bursa grandiflora has large flowers, as its name indicates, and it also has a balmy fragrance which I have not noted in any other species of *Bursa*. Both of these characters are important in relation to the fact that this species is self-sterile and that insect help is required, therefore, for successful seed-production (see fig. 14). It is the only self-sterile species known in the genus *Bursa*. The self-sterility results from the interplay of three or more allelic sterility factors s^1, s^2, s^3, etc., which seem to behave in essentially the same manner as those of *Nicotiana* (East 1925), *Veronica* (Lehmann 1927), and *Verbascum* (Sirks 1927). Not every cross is successful but as a rule only 50 per cent are compatible, the

remaining 50 per cent being no more effective than self-pollination. The capsules are usually a little under the average size for *B. bursa-pastoris*, and they are usually, but not always, somewhat concave-sided. The seeds are small. The leaves are rather thick and heavy, shining, with the lobes well separated (Fig. 14); the texture and general robustness of the plants almost suggesting tetraploidy,—which is found, however, not to be present, as will be indicated below.

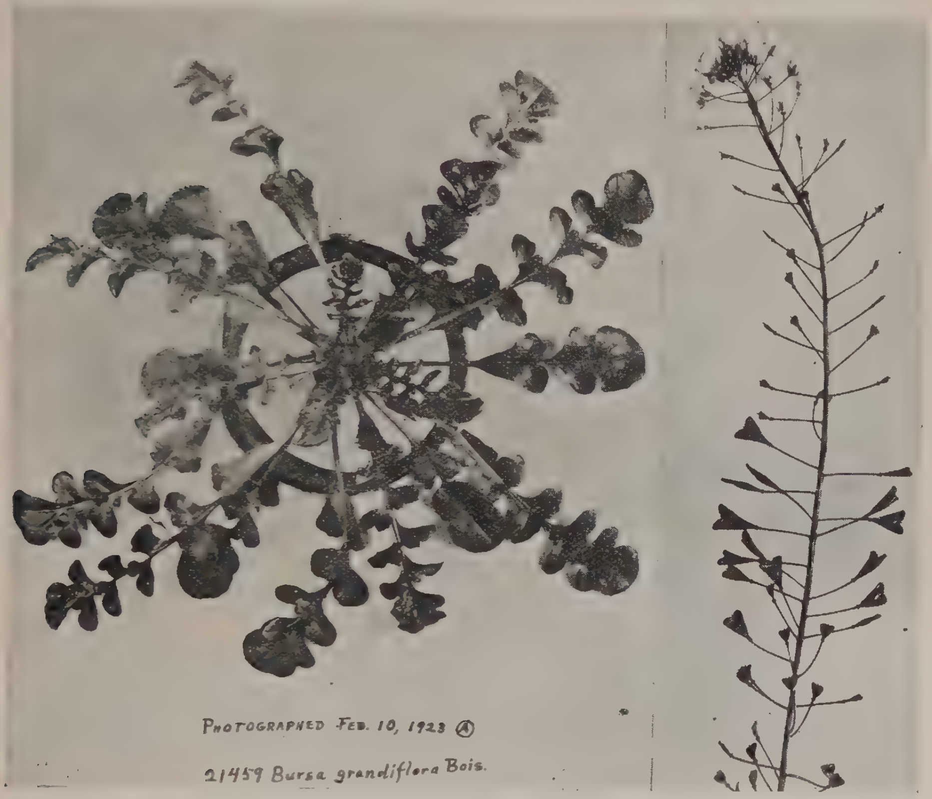

Fig. 14. Rosette and raceme of *Bursa grandiflora rhomboidea*. This species is self-sterile, and the cluster of developed capsules probably records a single visit of an insect bearing pollen from some compatible individual. Photograph by W. P. Agnew.

I have had two strains of this species, neither of which has come to me directly from a natural habitat. The first lot of seeds was received in 1914 from Doctor G. Bitter, Director of the Bremen Botanical Garden, without any data as to whence it had come. The second lot was received from the Brooklyn Botanic Garden in January 1925, the stock having come the preceding year from the Botanical Garden of Trieste, Italy, at the head of the Adriatic Sea. These two original cultures agree very well in all respects except that the rosette leaves of the Trieste strain have more highly developed *heteris* lobing, while the Bremen strain is usually *rhomboidea*-like, but with an occasional plant showing unmistakable *heteris* lobes. *B. grandiflora* is said to be common in

Greece and the Grecian Archipelago, and in fact it was first described by Boissier in 1842 from Corcyra, but the only collections of *Bursa* which I have yet received from Greece have been *B. bursa-pastoris*.

BURSA RUBELLA (REUTER)

Bursa rubella has small flowers, notably smaller than in *B. bursa-pastoris*, and rather small, concave-sided capsules with a shallow apical sinus. The central racemes usually show a long series of sterile flowers at the base. The rosettes are of pronounced *heteris* type and usually rather coriaceous in texture (Fig. 15).

Fig. 15. Rosette and raceme of *Bursa rubella* from Chiasso, Switzerland. The undivided leaves are juvenile, as all forms of rosette have the earlier leaves undivided. Photograph by W. P. Agnew.

The buds are commonly, but not always, strongly reddened, and the lunulae (rounded secondary lobes) of the leaf-lobes are in some strains brilliantly colored with reddish-purple anthocyan, though in other strains this reddening is likewise nearly or completely absent.

This species is abundantly distributed about the Mediterranean sea, and extends northward at least as far as central Switzerland and central France.

My first material of this species was received in July 1914 from Doctor H. W. Wollenweber, who collected it for me at Rome, Italy. A collection of

seeds received about the same time from Doctor G. Bitter, Bremen, under the name *B. rubella*, proved to be *B. bursa-pastoris*. I have since received *B. rubella* from Doctor Réné Maire, Algiers; from Vincenza, Italy, collected by Doctor G. Molon; from Pettorinello, Italy (Biagio Nini); from Chiasso, Switzerland (C. Schröter); from the Madeira Islands (T. D. A. Cockerell); and from Waco, Texas (Lulu Pace). This last collection, received in April 1919 from Doctor Lulu Pace, represents the first station for *B. rubella* in America, and so far as I am aware, this is still the only known station for *B. rubella* in the Western Hemisphere. Almquist's (1907, p. 15) reference to the occurrence of *Bursa rubella* and its relatives in Patagonia, Argentina, and other South American countries, undoubtedly rests on a misconception as to the nearness of relationship between *B. rubella* and *B. occidentalis*, two species which, on the genetical evidence, do not seem to be closely related to each other, notwithstanding their common possession of concave-sided capsules and frequently pronounced reddening of the lunulae of the leaves.

Fig. 16. Rosette of *Bursa Viguieri*. This has the genetical constitution of a *rhomboidea* rosette with the addition of a recessive inhibitor. The compactness of the rosette is associated with fasciation of the stems. Photograph by W. P. Agnew.

BURSA VIGUIERI (BLARINGHEM)

This is the most unique species of *Bursa* which has yet come to my attention. The flowers are very small, the capsules typically 4-carpellate, but ranging from 2 to 7-8-carpellate (Fig. 17). The central axis is always fasciated,

but usually grows only to a limited height and then gives rise to a number of lateral branches which are mostly not fasciated (Fig. 18). The rosettes are dense, the leaves being of inhibited *rhomboidea* form (Fig. 16), rugose and rather densely beset with very short, stiff, simple hairs, stellate hairs being very inconspicuous or absent.

B. Viguieri has been found only once in nature, having been discovered in 1908 by M. Paul Viguier 30 meters from the station of Izeste on the railroad from Pau to Laruns, Basses-Pyrénées, southwest France (Blaringhem and

Fig. 17. Raceme of *Bursa Viguieri* (right) compared with a raceme of *Bursa bursa-pastoris*. The fasciation is usually of the massive type and only rarely ribbon-like.

Viguier 1910, Blaringhem 1911). Only one specimen was found, and repeated collections at the same station covering a period of several subsequent years failed to discover additional specimens of *B. Viguieri*, but did show that *B. rubella* was the commonest species growing in that locality.

My material of *B. Viguieri* was received from Doctor Blaringhem in 1914 and has been grown every year since that time. It has proved with me, as it did with Doctor Blaringhem, to be completely constant.

BURSA TUSCALOOSAE N. SP.

This new species has capsules a little larger than in *B. rubella* with the lateral margins nearly rectilinear. The racemes usually show many sterile flowers

below, as in *B. rubella.* The rosette leaves are smooth, rather firm, dark green, with peculiar obtuse lobes curved conspicuously basad (Fig. 19). No red pigment has been noted on the leaves of this species. Seeds of *B. tuscaloosae* were first received in June 1918 from Professor J. Y. Graham of the University of Alabama. It has not yet come in from any other locality, but that it is an important component of the Bursa population at Tuscaloosa, Alabama, is de-

Fig. 18. Habit of *Bursa Viguieri.* Heavily fasciated below, but with lateral branches commonly not fasciated.

monstrated by the fact that 16 lots of seeds collected by Professor Graham in 1926 have yielded 12 families of *B. tuscaloosae* and 4 families of a rather striking type of dark-green *B. bursa-pastoris heteris.*

OTHER SPECIES

A futile attempt was made some years ago to secure material of the several species of *Bursa* which are recognized in the Kew Index as indigenous to Australia. Seeds labeled *B. humistrata* and *B. elliptica*, received in November 1918 from J. H. Maiden, New South Wales, Australia, failed to germinate.

Hymenolobus procumbens (L.) Nuttall (*Lepidium procumbens* L., *Capsella procumbens* Fries), which has often been referred by taxonomists to the genus *Bursa* (*Capsella*), has been received twice (July, 1916, and May, 1920) from Switzerland, through the kindness of Doctor C. Schröter. An extended search

Fig. 19. Climax leaves and raceme of *Bursa tuscaloosae*.

for this species made by a paid collector, in California, had failed to bring it to light in 1912, though it is included in the California manuals as a component of the flora of that state.

A careful consideration of the characteristics of this species (Fig. 20) together with many long-continued but wholly futile attempts to secure fertilizations between it and the other species of *Bursa*, have assured me that it is properly placed in a distinct genus. The long, thin delicate stems, leaves which give no

indication of the presence of any of the genetic factors which appear to be so generally distributed among the species of *Bursa*, the possession of a pungent flavor, flowers with a different form of petals and with the stamens differently arranged, and thin oblong capsules so unlike those of the various species of *Bursa* that they can not be brought into logical relation with the Bursa series,—all impress me with surprise that so many taxonomists have seen fit to include this species in the genus *Bursa* (*Capsella*). Briquet (1923) has recently discussed the taxonomy of this species at length and believes that it should be retained in the genus *Bursa* where it was placed by Fries (1832), but he has

Fig. 20. Rosette and capsules of *Hymenolobus procumbens* (L.) Nuttall (*Capsella procumbens* Fries). About natural size.

given no consideration to the genetic evidences that seem to make untenable its inclusion in the latter genus. If separated from the genus *Bursa*, as seems to be necessary on genetical grounds, it should become *Hymenolobus procumbens* (L.) Nuttall (1840).

Interspecific Hybridizations

As the consistent purpose of this study has been to analyze the genotypic constitutions of the several forms by separating out, and recombining in various ways, the separable elements of such genotypic constitutions, it will be readily

understood that the study of interspecific hybridizations, as such, has been to a certain extent incidental and secondary. The results recorded below are not in all cases, therefore, derived from crosses among the species as they have come in from nature, but often between forms possessing certain segregated characteristics of the wild species. As an example, the original *B. Heegeri* plants had the two dominant leaf-lobe factors *A* and *B*. After the first crosses had been consummated between this original *B. Heegeri heteris* and *B. bursa-pastoris simplex*, segregates were secured which had the *Heegeri* capsules associated with *simplex* rosettes. The great advantage of using a "tester" characterized by the four recessive factors *abcd*, as compared with the original *B. Heegeri* with factors *ABcd*, led to the continued use thereafter of the segregated *B. Heegeri simplex* in all crosses with new material in which a search for duplications of genetic factors was being made. Since different *Heegeri* segregates were used in different seasons, and even in different parts of the same season, it is conceivable that deviations in certain secondary effects of the hybridizations may be attributable to the existence of other differences in the genotypic constitutions besides the four recessive factors, *abcd*, which were being kept primarily in view in the crosses. As most of the results have been consistent, however, this source of possible discrepancies in the secondary effects of hybridizations should be kept in mind, but need not be given sufficient weight to invalidate any of the conclusions reached.

CROSSES OF BURSA HEEGERI WITH OTHER SPECIES OF THE FIRST GROUP

Since the primary objective of these investigations was to trace the geographical distribution of the duplication of factors for the characteristic triangular capsules, in the belief that such a study would throw light on the evolutionary history of the group, each new lot of *Bursa* material from any locality has first been crossed with *B. Heegeri*. Nearly two hundred of these original crosses have involved *B. bursa-pastoris* or its subspecies *apetala*, and have resulted in F_1 hybrids showing no sterility beyond what was normally present in the pure biotypes of *B. bursa-pastoris* or *B. Heegeri* used in the given cross, and have usually shown no deviation in any respect from typical *B. bursa-pastoris* having the same type of rosette as the wild parent. In other words, there is complete dominance of the *bursa-pastoris* characteristics over the *Heegeri simplex* characteristics, and no evidence of any disturbance whatever in the reproductive processes. In subsequent generations the segregations have sharply differentiated between the *bursa-pastoris* and the *Heegeri* characteristics, the F_2 showing an approximation to the 15:1 ratio, and the F_3 consisting of families of four kinds: (a) breeding true to *bursa-pastoris*; (b) segregating again as in F_2, 15:1; (c) segregating 3 *bursa-pastoris* : 1 *Heegeri*; and (d) breeding true to *Heegeri* (Shull 1914).

When subspecies *apetala* is crossed with *Heegeri* the behavior is the same as in other crosses between *B. bursa-pastoris* and *B. Heegeri*, except that the apetalous character proves to be a monomeric dominant, segregating out in the F_2 in a ratio of approximately 3 apetalous to 1 petaled (Dahlgren 1919).

With respect to rosette characters, the segregations in F_2 have given, *qualitatively*, the results expected on the assumption that *heteris* is produced by factors *AB*, *rhomboidea* by *aB*, *tenuis* by *Ab* and *simplex* by *ab*. Usually the ratios indicate that both *A* and *B* are each present in only one chromosome pair, but occasionally the *B* factor is found to be present in two chromosome pairs, giving rise to F_2 ratios of 15 *rhomboidea* to 1 *simplex* if the wild parent had *rhomboidea* rosettes; or 45 *heteris*:15 *romboidea*:3 *tenuis*:1 *simplex*, if the wild parent had *heteris* rosettes (Shull 1918). The capsule factors are inherited independently of the rosette forms.

In the F_3 from a cross of *B. Heegeri simplex* with a biotype of *B. bursa-pastoris rhomboidea* collected by Professor A. H. Trow in 1913, in Cathay Park, Cardiff, Wales, a new recessive *coriacea* factor, *k*, segregated out in a 15:1 ratio in addition to the factors *aB* of the usual rosette forms, and this *coriacea* factor has been used to test various biotypes of *B. bursa-pastoris*, the results usually demonstrating that its dominant allele, *K*, which produces the ordinary herbaceous texture of the leaves, is duplicated in two different chromosome pairs.

From several crosses between *Bursa Heegeri* and *B. bursa-pastoris*, and one cross between *B. Heegeri* and *B. orientalis*, all of which gave full fertility in the F_1, there has segregated in F_2 a definite Mendelian recessive type characterized by almost or quite complete pollen-sterility (Shull 1927). This pollen-sterile recessive has been repeatedly used in crosses with other species and biotypes, as it is very convenient for this purpose because no castrations are required. Occasionally the dominant pollen-fertility factor, S_p, is duplicated in *B. bursa-pastoris*, but more commonly it is monomeric.

When either *B. Heegeri* or *B. bursa-pastoris* is crossed with *B. occidentalis* the hybrids produced have the appearance of *B. bursa-pastoris*, the rosette being darker and of firmer texture than in *B. occidentalis*. The precocity of flowering is largely lost. The capsules of the F_1 hybrids are usually intermediate between *B. bursa-pastoris* and *B. occidentalis* (Fig. 21). When *B. Heegeri* has been used in the cross, the segregations in F_2 and subsequent generations indicate that the factor for inflated capsules is duplicated in *B. occidentalis* as it is in *B. bursa-pastoris*, and that there are in addition modifying factors which affect the depth and width of the apical sinus, curvature of the lateral margins and divergence of the angles of the capsules. The rosette-characters of the F_2 families are not classified quite so successfully as they usually are in crosses between *Heegeri* and *bursa-pastoris*, because of the relatively more frequent environmental modification of the lobing of the leaves, but usually there is evidence that the *B* factor in *B. occidentalis* is duplicated in two pairs of chromosomes (Shull 1918). The F_1 hybrids very commonly exhibit a considerable degree of sterility, though it has differed in different crosses, occasionally being indiscernible, and just as rarely, on the other hand, the sterility is practically complete. A striking form of semi-fertility has been frequently seen in which a succession of sterile and fertile phases follow each other on the racemes (Fig. 22). I am calling this most interesting form of reduced fertility "rhythmic

Fig. 21. Capsules of *Bursa occidentalis* (above), *B. bursa-pastoris* below, and the F_1 hybrid of these two species between. Each capsule was taken from a different plant.

Fig. 22. Fruiting racemes of *Bursa bursa-pastoris* (at left), *B. occidentalis* (at right) and the F_1 hybrid between, showing "proximal sterility" of *B. bursa-pastoris*, and "rhythmic sterility" in the hybrid.

sterility." A good example of this was shown in family 25411, produced by pollinating *B. occidentalis* by *B. bursa-pastoris apetala*.

Several F_1 families produced by crossing *B. occidentalis* or its subspecies *madeirae* on to the pollen-sterile segregates mentioned on page 861, consisted of all pollen-sterile plants (Fig. 23), while the same pollen-sterile type crossed with *B. Heegeri* or *B. bursa-pastoris* produce fertile F_1 hybrids, the pollen-sterile type reappearing in the F_2 and later generations. This cross may prove to be a useful criterion in any case of doubt as to the identity of a form which might perhaps be referred either to *B. occidentalis* or to *B. bursa-pastoris*.

Fig. 23. Rosette and raceme of the F_1 hybrid between a pollen-sterile segregate from (*Bursa bursa-pastoris*×*B. Heegeri*)F_x and *B. occidentalis*. All hybrids from this combination have been pollen-sterile, although pollen-sterility in crosses with *B. bursa-pastoris* is a recessive trait.

Bursa Heegeri crossed with *B. orientalis* produces F_1 hybrids which are usually as fertile as is *B. orientalis* itself, but in one cross (24363) between *B. bursa-pastoris* and *B. orientalis* the hybrids showed as pretty development of rhythmic sterility as any I have seen. In this case the *bursa-pastoris* was of the pollen-sterile strain. The rosettes showed the neat, well pronounced lobing of the *orientalis* parent, and the capsules likewise keep the broad, short form characteristic of *B. orientalis*. The F_2 segregation for capsule form shows the duplication of the factor for triangular capsules.

A cross made in 1915 between *B. orientalis* from Sapporo, Japan, and *B. bursa-pastoris apetala* gave an F_1 family (15580) which was almost completely sterile, but sufficient seeds were secured for the production of a second-generation family (16613), and these were mostly nearly fully fertile, though some sterile plants were also seen in the F_2. The difference of behavior between *apetala* and *Heegeri* in this cross suggests the possibility that *apetala* may have to be raised

to specific rank. It has not yet been used in a large enough series of combinations with other species to settle this question.

When *Bursa djurdjurae* is crossed with *B. Heegeri* the F_1 hybrids resemble *B. djurdjurae* in having shining, rather fleshy-appearing foliage with weak development of sinuses between the lobes. The stems also show the peculiar tendency to be erect below and procumbent above. The capsules are large, but not so conspicuously curved as in pure *B. djurdjurae*. The hybrids are as completely fertile as in the pure species. In the F_2 the segregation with respect to capsule form is 15 triangular to 1 round, showing the duplication of the inflation factor, and transverse curvature of the capsules is occasionally seen, but this character appears to be too obscure for definite genetical analysis.

Fig. 24. A "*simplissima*" rosette derived from a cross between *Bursa penarthae* and *B. Heegeri*. The factors for *rhomboidea* lobing are present, but can not manifest themselves because there is also present a recessive inhibitor.

The F_1 hybrids from a cross between *Bursa penarthae* and *B. Heegeri* were almost completely sterile, as were also the F_1 hybrids between *B. penarthae* and *B. bursa-pastoris*, but still a sufficient quantity of seeds was produced that subsequent generations could be analyzed. In the F_2 the usual 15 to 1 ratio with respect to capsule form indicated the duplication of the factor for triangular capsules. In minor details of form of the inflated (triangular) type of cap-

sule the influence of the basal taper of the capsules of *B. penarthae* was clearly seen, but in varying degrees, indicating the action of modifying factors. Most notable in the F_2 family was the appearance of a new recessive leaf-form, which is almost or quite free from lobing (Fig. 24). This form of rosette has been named *simplissima*, and has been proved to be determined by a recessive factor *i* which is an inhibitor of the lobing produced by other leaf-lobe factors, especially the factor *B*. When my strain of *simplissima* is crossed with a *simplex*, the F_1 hybrids are typical *rhomboidea*, showing that the *simplissima* may be represented by the formula *aaBBii* and the *simplex* by the formula *aabbII*. It is especially interesting that a cross between *simplissima* and *coriacea*, which latter likewise acts as a partial inhibitor of the leaf-lobing produced by the factor *B*, removes both of these recessive inhibitors temporarily from the field of action and gives in the F_1 only leaves of the usual lobing and texture of typical *rhomboidea*, for these two inhibitors are inherited independently of each other, so that the F_1 hybrid between them shows no influence of either inhibitor.

OTHER CROSSES BETWEEN SPECIES OF THE FIRST GROUP

While the crosses discussed in the last section, which had as one parent either *Bursa Heegeri* or *B. bursa-pastoris*, have been much more numerous than other combinations of species of the first group, several of the remaining possible combinations of these species have also been studied to a less extent. Thus, *Bursa occidentalis* fertilized by its subspecies *madeirae*, showed no reduction of fertility, and no new characteristics, the F_1 being typical *B. occidentalis*, and F_2 consisting of the P_1 types so overlapping that they could not be successfully separated by mere inspection. *Bursa occidentalis*×*B. djurdjurae* gave nearly complete fertility in F_1, the form and habit closely resembling the male parent, but usually showing no lateral curvature of the large capsules. *Bursa orientalis*×*B. djurdjurae* gave likewise a fully fertile F_1 hybrid family (25526) but in this case the putative hybrids closely resembled pure *B. orientalis*, and it may be that this result was due to a technical error, and that this family was in reality not hybrid but produced by the accidental self-fertilization of the maternal parent. *Bursa occidentalis madeirae*×*B. orientalis* (family 25394) gave an F_1 hybrid family mostly exhibiting no sterility, or with only a few sterile flowers at the bases of the racemes. The rosettes were more nearly as in *B. orientalis* and the capsules nearly as in *B. occidentalis*, but with the angles less divergent (Fig. 25). These and the remaining possible combinations between species of the first group need a much more extensive study, but it seems clear, at any rate, that the F_1 hybrids are generally fertile.

SPECIES OF THE SECOND GROUP CROSSED WITH BURSA HEEGERI AND B. BURSA-PASTORIS

Bursa grandiflora crossed with *B. Heegeri* or with *B. bursa-pastoris* has in many cases failed to set seeds, though the capsules usually enlarge to normal size. The contents of the capsules commonly show a number of brownish wafer-like ovules, which had apparently grown to about the full size of a devel-

oped seed and then aborted. I have made these crosses many times, using different biotypes of *Heegeri* and *bursa-pastoris*, including subspecies *apetala*, as pollinators, and have never secured a full set of good seed; but I have in several cases found a number of very small seeds, which obviously contained embryos, associated with the wafer-like aborted seeds, and from these I have grown about ten families including between 600 and 700 F_1 plants. These plants have been very vigorous and intermediate in appearance between *B. grandiflora* and

Fig. 25. Climax leaves and capsules of the F_1 hybrids between *Bursa occidentalis* and *B. orientalis* (left) and between *B. occidentalis* and *B. Viguieri* (right). The first, being a hybrid between two species of the *bursa-pastoris* group, is fertile, while the second is from a cross between a species of the first group and a species of the second group and is sterile.

B. bursa-pastoris. The flowers were large, but not quite so large as in *grandiflora*, and all had the pronounced balmy fragrance of *B. grandiflora*. Most of these F_1 hybrids were completely sterile, but in several instances one or two slightly enlarged capsules were found with one or at most two seeds in each capsule. By making a careful search for these rare seeds I succeeded in growing one F_2 family of 35 plants, but these were produced from unguarded F_1 plants and were again so completely sterile that it did not seem worth while to try to follow them further. The segregations with respect to capsule factors and leaf-

lobe factors could not be followed, and it could not be determined, therefore, whether any of the factors are duplicated or not. Dahlgren (1919, p. 58) also attempted a number of crosses of *Bursa grandiflora* with *B. Heegeri*, *B. bursa-pastoris*, and *B. b. p. apetala*, and his results, though rather meager, agree with mine. He mentions seven crosses which he carried out, but secured only one capsule with viable seeds, and this yielded three plants, two of which proved to be pure *B. grandiflora*, and the third had considerably smaller flowers and produced no capsules, the rudimentary capsules remaining undeveloped.

When *Bursa Heegeri* is used as a pollinator of *Bursa rubella*, a full set of plump seeds is usually secured. The F_1 hybrids are similar in form of rosette leaves to *B. rubella*, but a little more vigorous, less coriaceous in texture, with lobes a little less slender than in pure *B. rubella*. The flowering racemes become very long but rarely produce any good seed. I have repeated this cross nearly twenty times, and always with the same result. Just as in the cross between *B. grandiflora* and *B. Heegeri*, the F_1 hybrids, while usually completely sterile, rarely show a capsule slightly enlarged and containing one or two seeds, and by patiently searching for these I have been able to grow several later generations, but in such limited numbers and usually without guarding the pollinations, that I have not been able to make a satisfactory analysis of the differentiating characters. Several *Heegeri* and *Solmsiana* segregates have been found in these later generations, but all are still so sterile as to offer almost insuperable obstacles to their utilization in genetical experiments.

These sterile F_1 hybrids between *B. rubella* and *B. Heegeri* are identical with the F_1 hybrids between *B. Bursa-pastoris* and *B. rubella* of which also I have had two families. It will be recalled that this sterile hybrid has long been known in nature and has been repeatedly described in taxonomic works as *B. gracilis* (Grenier), and definitely assumed to be the hybrid between *B. bursa-pastoris* and *B. rubella*. So far as I can learn, however, such assumption had never been put to an experimental test until my crosses were made.

Crosses between *Bursa Viguieri* and *B. Heegeri* have been made several times and between *B. Viguieri* and *B. bursa-pastoris* many times, and always with the same results. A full quota of good seeds are produced from such pollinations and vigorous F_1 progenies are secured with great ease, but the F_1 plants are quite sterile and indistinguishable in any respect except the lobing of the rosette leaves from the hybrids of *B. rubella* and *B. bursa-pastoris* described in the last paragraph. Blaringhem (1911) secured the same result from repeated crossings between *B. Viguieri* and *B. bursa-pastoris* and drew the obvious conclusion that *B. Viguieri* is nearly related to *B. rubella*. Unfortunately he did not put this suggestion to the test by making a cross between *B. Viguieri* and *B. rubella* (see page 874).

The dominance of the *bursa-pastoris* characteristics in the hybrids of *B. Viguieri* and both *B. Heegeri* and *B. bursa-pastoris*, is complete, as the F_1 plants show no trace of the supernumerary carpels, fasciated stems, rugose foliage and inhibited lobing of the leaves, which so strikingly differentiate *B. Viguieri* from all other species. When the *bursa-pastoris* or *Heegeri* parent has the *simplex*

type of rosette, the F_1 hybrids always show typical *rhomboidea* rosettes, thus demonstrating that *B. Viguieri* possesses the leaf-lobe factors *aB* plus a recessive inhibitor, *i*, which nearly prevents the *B* factor from expressing itself. Just as was noted above in the sterile hybrids of *B. grandiflora* and *B. rubella* with

Fig. 26. A fruiting raceme of the F_1 hybrid between *Bursa tuscaloosae* and *B. Heegeri*, which is completely sterile. Photograph by W. P. Agnew.

B. Heegeri and *B. bursa-pastoris*, these sterile hybrids of *B. Viguieri* very rarely produce a slightly enlarged capsule with one or two good seeds, but not enough to justify an attempt to make a further genetical analysis by the use of these rare seeds.

In 1915 a cross was made between *B. bursa-pastoris apetala* and *B. Viguieri* which gave an F_1 family (15578) in full agreement with other crosses between *B. Viguieri* and *B. bursa-pastoris*, except that in this case all the hybrids were approximately apetalous, the four staminodes having the upper half of the anthers petal-like, the lower half anther-like. These four staminodes reached the tips of the sepals while the true stamens remained considerably shorter. All were completely sterile.

Bursa tuscaloosae crossed with *B. Heegeri* or *B. bursa-pastoris* produces an abundance of good seeds, and the F_1 hybrids have been produced easily in large numbers, but the hybrids are sterile (Fig. 26) and differ from the sterile hybrids of the preceding three crosses only in having rosette leaves which display some basad curvature of the primary lobes like that seen in pure *B. tuscaloosae*, but this curvature is less pronounced in the F_1 hybrids than in this pure species.

Fig 27. Climax leaves and fruiting racemes of the F_1 hybrid between *Bursa occidentalis madeirae* and *B. rubella* (left) and between *B. occidentalis* and *B. tuscaloosae*. Both are completely sterile.

CROSSES OF OTHER FIRST-GROUP SPECIES WITH SPECIES OF THE SECOND GROUP

Bursa occidentalis has been used many times to pollinate *B. grandiflora* and the reciprocal cross has also been made several times, but only one hybrid seedling has been secured, and this plant, which had nearly the aspect of *B. occidentalis* in the rosette stage, was entirely sterile. Three attempted hybridizations between the subspecies *madeirae* and *B. grandiflora* have likewise

produced just one sterile hybrid. Crosses between *B. occidentalis* and the remaining three species of the second group have been more successful: Seven crosses with *B. rubella* have yielded seven thrifty F_1 families with a total of 716 plants which have been entirely sterile; and the subspecies *madeirae* crossed with *B. rubella* has produced three families of completely sterile hybrids, comprising 337 individuals (Fig. 27). With *B. Viguieri*, *B. occidentalis* has given four F_1 hybrid families, with a total of 414 individuals all completely sterile, except that once I found a slightly enlarged capsule containing two seeds, and grew two plants from these, but lost them through an accident before I had observed their condition as to fertility or sterility. *Bursa occidentalis* has also been crossed with *B. tuscaloosae* five times, yielding F_1 hybrids with a total of 368 plants, all completely sterile (Fig. 27), while two families from crosses between *B. occidentalis madeirae* and *B. tuscaloosae* have given 95 F_1 hybrids likewise completely sterile.

Bursa djurdjurae has been crossed several times with *B. grandiflora* usually without securing viable seeds, but once (family 15582) an F_1 progeny was secured, consisting of 93 plants. These had rather low-grade *rhomboidea* rosettes and the large flowers and balmy fragrance of *B. grandiflora*, but were all seemingly sterile. Owing to the fact that *B. grandiflora* is self-sterile, it was deemed advisable actually to test the sterility of these plants by hand-pollinations. Good pollen was obviously lacking but the stigmas appeared quite normal. Four of these series of hand-pollinations were made: (a) 37 flowers castrated[2] and supplied with pollen of *B. Heegeri* yielded a single seed; (b) 40 flowers castrated and crossed with *B. djurdjurae* were wholly without noticeable effect. (c) 40 flowers castrated and treated with pollen of *B. grandiflora* showed no capsule more than slightly enlarged, and containing not a single seed; (d) 89 flowers castrated and crossed with another *B. grandiflora* possessing a different self-sterility factor, likewise resulted in no fertilization. On several other plants single capsules were found each of which contained one seed. An F_2 progeny of three plants was grown from these seeds (Fig. 28), and as one of these showed considerable fertility, and the others produced a few seeds, two F_3 progenies and 7 F_4 progenies were grown, but there seemed to be no further increase in fertility, and the line was dropped at the close of the fourth generation. *B. djurdjurae* has been mated once with each of the other species of the second group. With *B. rubella* it gave 124 F_1 hybrids; with *B. Viguieri* 10 F_1 hybrids were secured, and with *B. tuscaloosae* 97 hybrids were produced, plus one fertile plant of the maternal type which resulted from an accidental self-fertilization. All of these hybrids were completely sterile, and were also notable because of the conspicuous effect of the *B. djurdjurae* parent in reducing the lobing of the rosette leaves, especially in the cross with *B. rubella*, which has high-grade *heteris* leaves, the hybrid being scarcely recognizable as a *heteris*.

Bursa orientalis, crossed several times with *B. grandiflora*, produced fully

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[2] This consisted merely in removing the floral envelopes so as to facilitate pollination. There were no good stamens to remove, of course.

enlarged capsules but very few viable seeds. Only two F_1 hybrids have been secured and these were completely sterile. *B. orientalis* has not yet been used in a cross with *B. rubella*, but with *B. Viguieri* one cross (family 23237) yielded 89 completely sterile hybrids which became mixed with pure *orientalis* through an unfortunate delay in collecting the seed, which allowed an uncastrated side-branch to develop and ripen seeds in the bag with the hybridized seeds. Twenty-six of these pure *B. orientalis* were included in family 23237. They were recognized from early in their development, and they were, of course, in contrast to the hybrids, fully fertile. One attempt has been made to cross *B. orientalis* with *B. tuscaloosae*, but this failed because the inflorescence which was used for

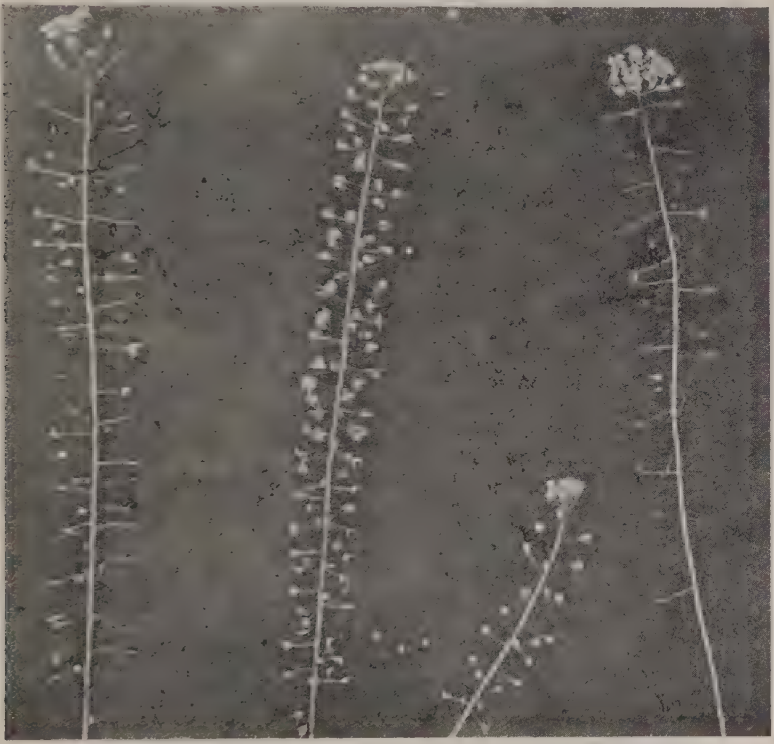

Fig. 28. Fruiting racemes of three F_2 hybrids between *Bursa grandiflora* and *B. djurdjurae*. These hybrids were almost but not quite completely sterile.

the cross blighted before the seeds could reach their full development. There is no reason to believe that this combination can not be easily accomplished if further attempts be made.

CROSSES BETWEEN SPECIES WITHIN THE SECOND GROUP

Bursa grandiflora × *B. Viguieri*. After having made over twenty different crosses between *B. Viguieri* and as many various biotypes of *B. bursa-pastoris* or *B. Heegeri*, and found their F_1 hybrids sterile, and after having attempted

to make about the same number of crosses in which *B. grandiflora* was combined with *B. bursa-pastoris* or *B. Heegeri*, but which had given hybrids only rarely, and such as were secured were completely sterile, I was greatly astonished when on May 29, 1918, I discovered that two crosses which I had made the previous year between two specimens of *B. grandiflora* and a *B. Viguieri were conspicuously fertile!* This result was the more surprising from the fact that these two species stand at the opposite extremes of *Bursa* variation, with *B. bursa-pastoris* practically midway between them (Shull 1923). The F_1 plants had nearly the vegetative aspect of *B. grandiflora*, showing no fasciation. The flowers

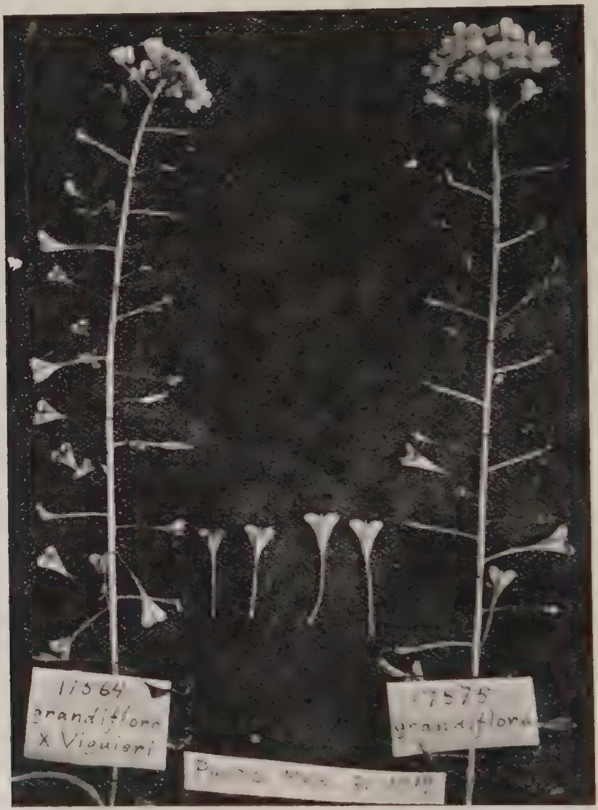

Fig. 29. Racemes and capsules of *Bursa grandiflora* (right) and the F_1 hybrid between *B. grandiflora* and *B. Viguieri*. All the peculiar characteristics of *B. Viguieri* have disappeared, giving a hybrid resembling *B. grandiflora*, except that it is fully self-fertile.

were intermediate in size between those of the two parents but had the peculiar fragrance of *B. grandiflora*; the capsules were generally bicarpellary with concave lateral margins (Fig. 29). On several of the plants one to several tricarpellary capsules were seen at the base of the central raceme, but not more than are sometimes seen on pure *B. grandiflora*. All of the peculiar characteristics of *B. Viguieri* were lost except the capacity for self-fertilization, but all of

the "lost" characteristics were recovered in the F_2 families and thus was made possible a genetical analysis of many of the characteristics of *B. Viguieri* to which Blaringhem had devoted so many futile attempts and for which I had also worked for several years in vain.

The detailed results of the extensive series of experiments involving the F_2 to F_8 and later generations from these crosses must be reported in a special paper, but it may be stated that the supernumerary carpels and fasciated stems of *B. Viguieri* segregate out together as a recessive trait in a monohybrid Mendelian ratio, that the self-sterility of *B. grandiflora* does the same, and that the leaf-characters of *B. Viguieri* are produced by three Mendelian factors,

Fig. 30. Fasciated bicarpellary segregates from the cross between *Bursa grandiflora* and *B. Viguieri*. Note that the fasciation is of a different type from that in *B. Viguieri*.

a and *B* of *rhomboidea* together with a recessive inhibiting factor, i_2, which nearly suppresses the *rhomboidea* lobing. This inhibiting factor is independent of the other leaf-factors, and is also independent of the fasciation factor which causes not only the thickening of the stem but also the denseness of the rosette and the addition of the extra carpels. Several additional characteristics have been derived which indicate that there is a factor for revolute leaf-margins in *B. grandiflora*, and a second fasciation factor in *B. Viguieri*, which is capable of separation from the main fasciation factor mentioned above, so that a fasciated

strain with bicarpellary carpels can be derived without the occurrence of a break between the association of supernumerary carpels and the usual type of fasciated stems (Fig. 30).

Fig. 31. Rosette (above) and fruiting raceme of the F_1 hybrid between *Bursa rubella* and *B. Viguieri*. Below is a rosette of pure *B. rubella* derived from Waco, Texas. In nature, this hybrid would hardly be distinguished from *B. rubella*, but the lobing of the leaves is less slender and less sharp, and the leaf-surface exhibits some of the rugosity characteristic of *B. Viguieri*. Photograph by W. P. Agnew.

Bursa rubella×*B. Viguieri*. In 1921, after the preliminary demonstration that *B. rubella*, like *B. grandiflora* and *B. Viguieri*, gives only sterile hybrids with *B. Heegeri* and *B. bursa-pastoris*, it was recognized as desirable to make crosses between *B. rubella* and these other two species, on the assumption that similarity of behavior in crosses with *B. Heegeri* and *B. bursa-pastoris* indicated a close relationship of *B. rubella* to *B. grandiflora* and *B. Viguieri*. There was

the further suggestion of such a relationship in the fact that all three of these species have relatively small, mostly concave-sided capsules. The pollination of a *B. rubella* derived from its only known American station at Waco, Texas, with pollen of *B. Viguieri* gave in 1922-1923 a family (No. 21413) consisting of a mixture of 43 pure *rubella*, produced by accidental selfing of the mother plant, and 194 F_1 hybrids which differed from the pure *rubella* in having the primary lobes of the leaves broader, the pubescence of fine, straight, simple hairs as in *Viguieri*, and the stems 20 per cent longer. These hybrids were completely fertile (Fig. 31), and have given rise to a series of families of F_2 and later generations in which the segregations have served to confirm the conclusions drawn from the cross between *B. grandiflora* and *B. Viguieri*, as to the genetical constitution of the latter species. One of the *rubella* plants of family 21413 was in turn pollinated by *B. Viguieri* and gave in 1923–1924 a uniform progeny (family 22360) of 170 completely fertile F_1 hybrids having the same character as the supposed hybrids of family 21413, and the F_2 progenies have shown the same segregations, which have been carefully studied.

Bursa grandiflora×*B. rubella*. In the winter of 1921–1922 several crosses were made between these two species. One of these resulted from pollination of a *B. grandiflora rhomboidea* of my Bremen strain with pollen of *B. rubella* derived from Chiasso, Switzerland. This gave an F_1 family of 16 plants (family 21414) having the heavy texture of *B. grandiflora*, but showing unmistakable effects of the *heteris* lobing of *B. rubella*. The flowers were less large than in *B. grandiflora* though much larger than in *B. rubella*, being slightly larger than in *B. bursa-pastoris*, and the characteristic balmy fragrance of *B. grandiflora* was very much reduced. The most remarkable deviation from the *grandiflora* mother, however, was the fact that these 16 plants *were completely self-fertile*, as in *B. rubella* (Fig. 32).

The second cross was made by using pollen of *B. rubella* from Waco, Texas, which differed from the Chiasso strain in having much more pronounced red pigmentation on the median portions of the rosette leaves. The F_1 family (21415) consisted of 117 plants, which agreed with the F_1 of the previous cross, except in having more red pigmentation on the lunulae of the leaves. All of these were likewise completely fertile.

In 1925 *B. grandiflora* was again crossed with *B. rubella* from Waco, Texas, and yielded in 1926 an F_1 progeny of 21 plants which agreed with those of the previous cross in being fully fertile, except for the occurrence of sterile flowers at the base of the central raceme, which are usually present also in pure *B. rubella*.

A reciprocal hybridization was also attempted in 1921 by applying the pollen of *B. grandiflora* to castrated flowers of *B. rubella* from Waco, Texas. Only one F_1 plant was produced from this cross (family 21306) and it agreed with the reciprocal F_1 hybrids and was, like them, abundantly self-fertile.

An F_2 family (22493) and several F_3 families were derived from this last F_1 hybrid and gave a sharp segregation into *heteris* and *rhomboidea* and into self-fertile and self-sterile plants, but *no hybrid sterility* appeared. The self-sterile

plants as well as the self-fertile ones produced perfectly good fertilizable eggs and completely potent sperm, they were merely incapable of pollinating themselves or each other, but could be easily crossed with appropriate testers of *B. grandiflora* or with any plant of a self-fertile species.

Bursa rubella×*B. tuscaloosae.* A cross between these two species produced in 1925-1926 an F_1 family (24344) consisting of 123 plants of which 13 proved to be pure *B. rubella*, due to accidental self-fertilization, and 110 hybrids which

Fig. 32. Rosette and fruiting raceme of the F_1 hybrid between *Bursa grandiflora* and *B. rubella*. This hybrid is not only fully fertile but also fully self-fertile, showing the dominance of self-fertility over self-sterility. Photograph by W. P. Agnew.

could be recognized as hybrids by being less high-grade *heteris* than in pure *rubella*, and by showing in some of the proximal lobes of the leaves the characteristic basad curvature of *B. tuscaloosae* (Fig. 33). These hybrids were not completely fertile, but still were fertile enough that an abundance of seeds were secured by merely placing bags on the F_1 plants. Two large F_2 families have been grown from self-fertilized F_1 hybrids, but these have not yet been examined.

Bursa grandiflora×*B. tuscaloosae.* This combination was made in 1925 and the F_1 family (24340) consisted of only two plants, which were vigorous specimens of rather low-grade *heteris* with some of the primary lobes, especially near

the middle of the climax leaves, showing a slight recurvature suggestive of *B. tuscaloosae* (Fig. 34). The flowers were large and showy and with the characteristic balmy odor of *B. grandiflora*. They were completely self-fertile from the first flower onward. Two F_2 families (25439, 25440) have been grown in 1926-1927 and show a sharp segregation to self-fertile and self-sterile groups, but no segregate has failed to produce good eggs and good sperms. The leaf segregations have not been sufficiently definite to permit of classification into the P_1 types, meaning probably that more than one factor-difference is concerned.

Fig. 33. Climax and post-climax leaves and capsules of *Bursa rubella* (left), *B. tuscaloosae* (right) and their F_1 hybrid. The photograph exhibits some effect of heterosis. The hybrid is completely fertile.

Other combinations within the second group. Various combinations have been made involving segregates from certain of these foregoing crosses, with other species of the same group. Thus, segregates from *grandiflora* × *Viguieri* have been crossed again with *grandiflora*, *Viguieri*, and *rubella* (Fig. 35); segregates of *grandiflora* × *rubella* have been crossed with *tuscaloosae*; and *grandiflora* has been crossed with segregates from *B. rubella* × *Viguieri*, and all of these combinations have produced fertile offspring.

On the other hand, many of these segregates have also been crossed with *B. Heegeri*, of the first group, and have then given only sterile progenies (Fig.

35). I have not been able to break down the barrier which separates the two groups.

The Significance of the Two Groups of Species

The genetical results show that the species which have been involved in these cultures divide sharply into two groups, the intra-group crosses producing fertile hybrids (partially sterile in some cases), while all the inter-group crosses produce hybrids which are practically completely sterile. The morphological differences between several of the species of the second group are greater than the difference between *B. rubella* or *B. tuscaloosae* on the one hand, and several

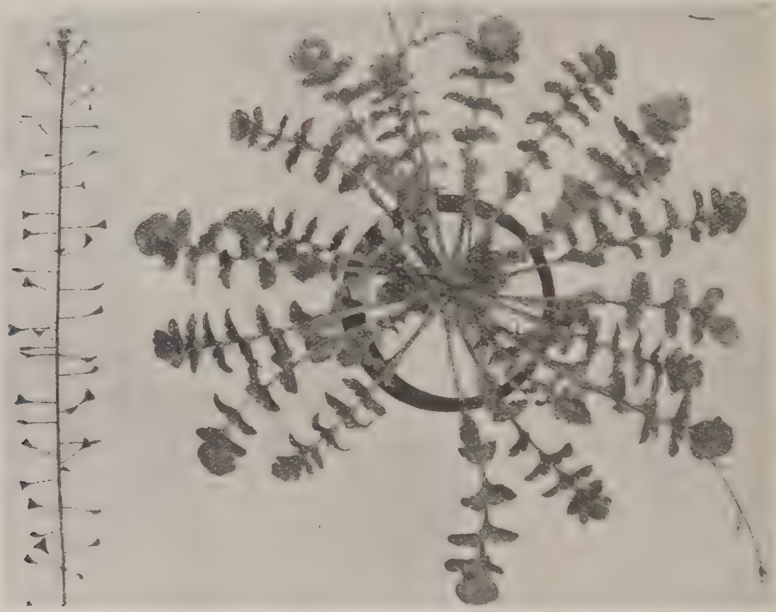

Fig. 34. Rosette and fruiting raceme of the F_1 hybrid between *Bursa grandiflora* and *B. tuscaloosae*, showing a backward curvature of the primary leaf-lobes, which, along with complete self-fertility, were derived from the pollen-parent, *B. tuscaloosae*.

of the species of the first group, on the other. What, then, is the fundamental difference between the two groups which accounts for the sharp difference in genetical behavior? Fortunately, we are now in position to answer this question. Rosenberg (1904) reported that the numbers of chromosomes in *Bursa bursa-pastoris* are 16 haploid, 32 diploid, and about 48 in the $3n$ nuclei of the endosperm, and Laibach (1907) confirmed these counts. In 1920 Marchal gave the count for *B. Heegeri* as 16 haploid, the same as *B. bursa-pastoris*, but reported that the number in *B. Viguieri* is just half this,—8 haploid. This report of Marchal was recognized at once as giving the probable answer to the riddle of the two groups, and this answer has now been fully confirmed by one of my students who has demonstrated that the species of the first group with the ex-

ception of *B. penarthae*, of which no material has been available for cytological study, are all characterized by 16 haploid chromosomes, and that all four of the species of the second group have 8 haploid chromosomes (Hill 1927).

Perhaps the most surprising feature of these cytological discoveries is that *B. grandiflora* with its rather stiff, fleshy, shining foliage and large flowers, and *B. Viguieri* with its dense rosettes, heavy, fasciated stems and supernumerary carpels, are diploid species, while *B. bursa-pastoris* and *B. occidentalis* with their

Fig. 35. Racemes from two triple crosses. At left, *Bursa* ((*grandiflora* × *Viguieri*)F_4 × *rubella*)F_1; at right, *Bursa* ((*grandiflora* × *Viguieri*)F_1 × *Heegeri*)F_1. In the former, all three species are in the *rubella* group; in the latter, two species are in the *rubella* group, but the third is in the *bursa-pastoris* group. Photograph by W. P. Agnew.

more tender, pliant foliage, slender stems and rather small flowers are tetraploids, or preferably "double diploids" (Blakeslee, Belling, and Farnham 1923), when analogy with other genera in which diploid and tetraploid species occur might well suggest the reverse relation between the two groups, at least with respect to the four species here placed in contrast with each other.

This explanation of the two groups serves to bring the sterile hybrids into line with a whole class of previous experiences, for these hybrids are triploids, and triploids are notoriously defective in gamete formation. The question arises at once whether other sterile hybrids may not be rather generally the result of unbalanced chromosome numbers. Many cases are known in which this is true, but the question can not be adequately discussed here.

Discussion

The attempt which is here made to distinguish several species within the groups of forms which have been quite commonly treated as single variable species, will doubtless meet with various degrees of approval or of disapproval from taxonomists. The fact that certain of these species are differentiated from others by having different chromosome numbers will undoubtedly be accepted by all as necessitating the recognition of more than one species within the group of forms here dealt with, even though some of the proposed new species may not seem to all to be differentiated by sufficiently conspicuous characteristics to justify their recognition as valid species.

The prime consideration which has led me to propose these species has been the practical one that the recognition of these species makes it easiest to give a cogent account of the genetical experiments which are in progress on this genus. There can be no doubt that many other species of *Bursa* exist in nature which are only awaiting discovery, but this does not mean that *almost any plant* that may be picked up will be a new species. In the United States I have had numerous collections representing nineteen states, but all have thus far been *B. bursa-pastoris*, except at Waco, Texas, where *B. rubella* is mixed with *B. bursa-pastoris;* at Tuscaloosa, Alabama, where *B. tuscaloosae* is associated with *B. bursa-pastoris*; and from Tucson, Arizona, westward along the Mexican border and in California, where, thus far, only *B. occidentalis* has been sent to me.

I have given some thought to the question of priority of Almquist's names of elementary species, but have decided to retain the names which have been in use in my notebooks. It is probable that *C. b. p. concava* Almquist is the first published name which applies to material of my *Bursa occidentalis*, but also that his *C. b. p. patagonica* belongs to the same species, though probably to a different biotype. My designation of this species as *B. occidentalis* should leave Almquist's names unchanged in significance, so that his *C. b. p. concava* would become *Bursa occidentalis concava*, and so on.

While the recognition of these species has in the first instance rested upon genetical experiments, I am not proposing that the *identification of individuals* of *Bursa* should in future require genetical testing. All of these species are characterized by sufficiently obvious macroscopic features that their identification will involve few errors by those who have learned their significant features.

The important contribution which genetics can make and should make to taxonomy has to do with the more fundamental philosophical problem of finding the *natural relationships among the taxonomic groups*. Ever since the first

crude attempts to arrange species into *natural* groups, taxonomists have assumed that such grouping represents significant *genetical* connections. Even Linnaeus himself supposed that all of the individuals of any species are descended from a common ancestor which received its assemblage of distinguishing characteristics from the hand of the Divine Creator, and that these characteristics have been simply repeated, with only minor deviations, through the numerous generations which have come and gone since the great day of such specific origins. Since the finding of natural relationships between species is generally recognized as a genetical problem, it should be, wherever possible, attacked by genetical methods.

Assumptions of genetic relationships based on a study of phenotypic resemblances may easily be in error, owing to the fact that such resemblances are not always the result of genetic continuity. For example, when Solms-Laubach (1900) undertook to determine the relationship of *Bursa Heegeri*, his first impression, based upon the round, uninflated capsules, was that he had before him a species of some genus near to *Camelina*. Only when his *cultures* showed specimens with "reversionary" capsules, essentially identical with those of *Bursa bursa-pastoris*, did he correctly refer this species to its true generic relationship. Even without the reversionary capsules, he could have arrived at the same conclusion, had he hybridized *B. Heegeri* with *B. bursa-pastoris*, since in this cross there is no disturbance of the reproductive processes.

Almquist (1907, p. 15; 1926, p. 45) may be right, that *Bursa occidentalis* is more closely related to *B. rubella* than are the numerous biotypes of *B. bursa-pastoris*, but the hybridization phenomena presented in this paper show that such relationship is in reality rather remote and that the present constitution of *B. occidentalis* is closer to that of *B. bursa-pastoris*, to *B. Heegeri*, and to *B. orientalis*, than it is to *B. rubella*. From all points of view it seems that *B. rubella* is nearest to the stem-form from which all of the species which make up the genus *Bursa* have been derived, but whether *B. bursa-pastoris* has been derived from *B. occidentalis*, or whether it was produced directly from some as yet undiscovered species of the *rubella* group, can not be determined at present. If an 8-chromosome species should be found that resembles as closely the usual biotypes of *B. bursa-pastoris* as *B. occidentalis* resembles *B. rubella*, it would be plausible to assume that two independent tetraploid mutations produced the *bursa-pastoris* group of biotypes and the *occidentalis* group. In the absence of any evidence for the present existence of such a putative pre-*bursa-pastoris* species, speculation as to the evolutionary relationship of these two groups is of little value.

The relationships among the four species of the *rubella* group also present difficulties, chiefly because of the wide differences between these species. Blaringhem's (1911) conclusion that *B. Viguieri* arose by mutation from *B. rubella* was a natural one from the single genetical fact which he had before him, namely, that the hybrids between *B. Viguieri* and *B. bursa-pastoris* are indistinguishable from *B. gracilis* (Gren.) which had long been recognized as the sterile hybrid between *B. rubella* and *B. bursa-pastoris*. Had he followed up

this suggested relationship between *B. rubella* and *B. Viguieri* by making crosses between them he would have found, as I have, that these two species differ from each other in three Mendelian factors of major importance and in several additional factors of less importance. That such a complex assemblage of independently inherited genetical differences could have originated by a single mutation is, of course, quite out of the question. Blaringhem (1911, p. 287) mentions the possibility that the single individual of *B. Viguieri* which was found on the railroad embankment at Izeste, Basses-Pyrénées, may have been transported thither from some other point, but adds that so far as he knows no analogous form has ever been reported either from the Basses-Pyrénées or elsewhere. The demonstration that this unique species can not conceivably have arisen by a single mutation directly from *B. rubella*, its nearest known relative, strengthens the view that 1908 at Izeste was neither the time nor the place of its origin, but that its evolution has involved a relatively long series of mutative steps. It may be confidently predicted that a more extensive exploration will bring to light somewhere, if not *B. Viguieri* itself, at least several elementary species, now unknown, which will differ from *B. rubella* on the one hand and from *B. Viguieri* on the other, by single Mendelian factors and which will complete the chain of single steps necessary to be traversed in passing from one of these species to the other. There should be a *rubella*-like species with *rhomboidea* rosettes, and probably also one with the inhibitor which nearly prevents the *B* factor from manifesting the peculiar *rhomboidea* type of lobing, or possibly the inhibitor may have appeared before the change to *rhomboidea*, giving a *rubella*-like species with inhibited *heteris* rosettes. Almquist's (1907, pp. 18, 19) elementary species, *C. b.p. Reuteri*, which he secured in 1904 from the botanical garden at Besançon, France, may represent this combination, as it is described as having the rosette leaves "lange Zeit ganzrandig, aber schliesslich unregelmässig eingeschnitten." Almquist's figure 10, representing a winter rosette of this elementary species, shows a rosette which might be appropriately described as an "inhibited *heteris*." This rosette could have been practically duplicated in some of the F_2 segregates from the cross between *B. rubella* and *B. Viguieri* in my cultures. It has long been known that there is no logical sequence of occurrence of mutations and it is not possible to say *a priori* in what order the several steps were taken which led from *B. rubella* to *B. Viguieri*, as the latter species is characterized today. The most important mutation of all, the one which produced the fasciated stems and tetracarpellate capsules, may have been the first step just as easily as the last step in the series. If it was the first step, there resulted a *Viguieri*-like species with normal *heteris* rosettes unmodified by an inhibitor, thus agreeing with the rosette of *B. rubella*. This combination of fasciated stems with typical *heteris* rosettes has also been exemplified by segregates from my crosses of *B. rubella* with *B. Viguieri*.

On discovering that there are duplicated Mendelian factors which differentiate between the triangular capsules of *B. bursa-pastoris* and the top-shaped capsules of *B. Heegeri*, I offered several suggestions as to how such duplication might conceivably have taken place (Shull 1914, 1918). All of these suggestions

were based on the tacit assumption that the number of chromosomes had remained unchanged when the duplication of factors occurred. Marchal's (1920) discovery that *B. Heegeri* has 16 haploid chromosomes as in *B. bursa-pastoris* (Rosenberg 1904, Laibach 1907), and that *B. Viguieri* has only 8 haploid chromosomes, put an entirely different face on the problem; for it suggested at once that the duplication of factors in *B. bursa-pastoris* probably resulted from an original duplication of whole chromosomes as a result of a tetraploid mutation, and also that, as a corollary, the 8-chromosome group (the *rubella* group) should be recognized as the ancestral group, from which the *bursa-pastoris* group has been derived. The problem of the origin of duplication of factors seems thus to be settled, but its settlement on the basis of the duplication of the entire set of haploid chromosomes raises several reciprocal problems: Why should not every recessive trait when crossed with a corresponding dominant result in each case in a 15:1 ratio in the F_2? Thus far, the only traits which have approximated this condition have been the *Heegeri* type of capsule and the *coriacea* type of rosette. The B factor which produces the deep leaf-sinuses of *rhomboidea* and *heteris* and the S_p factor which makes possible the development of good pollen, *are usually not duplicated*, though sometimes they are duplicated. The B factor seems to be generally duplicated in *B. occidentalis*, but only occasionally in *B. bursa-pastoris*. The A factor which produces the elongation of the leaf-lobes characteristic of the *heteris* and *tenuis* types of rosette have shown no indication of ever being duplicated.

There is a still more fundamental problem, which was pointed out by Blakeslee, Belling and Farnham (1923), namely, How can the tetraploid mutation produce a "double diploid" condition in which four homologous chromosomes form two definite disomes instead of a single tetrasome?

I have no experimental basis at present for a solution of these problems. The occurrence of "loss" mutations in the B and S_p factors, or any other factors which are not now duplicated, may be suggested as possibly a partial solution of the first problem; but whether other mutations taking place in the tetrasomes can in some way bring about their assortment into definite pairs, thus giving rise to the observed 15:1 ratios instead of the 35:1 ratios theoretically expected in tetrasomic inheritance, is not now apparent. There have been in some cases considerable deviations from the 15:1 ratio between triangular and round capsules, and usually in the direction of the tetrasomic ratio, but I am not prepared at present to attribute any of these deviations to the occasional mating of chromosomes of one disome with the members of another disome derived from the same original tetrasome. If a goodly number of linkage relations could be established, this question could probably find an experimental solution, but only three cases of linkage in *Bursa* have been clearly established as yet, and none of these involves the *Heegeri*-type of capsule. The leaf-lobe factors are generally too uncertain in their expression to be very satisfactory for use in solving a critical problem of this kind.

In agreement with the conclusion that duplication of factors has resulted from the tetraploid mutation (or mutations) by which the *bursa-pastoris* group

was derived from the *rubella* group, is the fact that no duplicated factors have yet been found in the *rubella* group, but fewer factors have been studied in the *rubella* group, and the experiments have not yet been carried out on such an extensive series of biotypes as have those which have had to do with the *bursa-pastoris* group. Consequently, too much stress must not be laid on the absence of evidence of duplication in the *rubella* group.

The importance of polyploidy as an evolutionary process has been remarked by many authors and will not be discussed at length here. It may suffice to point out several correlations in *Bursa* which are in keeping with observations made on certain other polyploid species, and which I believe to be of fundamental significance. The *rubella* group of species, although older than the *bursa-pastoris* group, exists in a relatively much smaller number of biotypes, and these are mostly very local in distribution, being limited, so far as now known, to the region immediately surrounding the Mediterranean Sea, and a small part of the Southern states of the United States. Perhaps the small-capsuled forms from Siberia, mentioned by Almquist, may belong in the *rubella* group. The *bursa-pastoris* group, on the other hand, has produced an enormous number of biotypes which occupy not only all of the regions in which the *rubella* group is found, but all of the rest of the temperate regions of the world as well. The greater number of chromosomes has evidently given greater variability, greater adaptability, greater vigor and greater hardiness.

The recognition of two groups of species having different chromosome numbers and therefore differing in their hybridization relations gives a simple explanation of Almquist's conclusions concerning the unequal frequency of occurrence of hybridizations among the different *Bursa* species in his cultures. He reports (Almquist 1926, p. 44) (a) that hybrids are scarcely ever found among the Swedish forms, either in nature or in his cultures; (b) that *Bursa grandiflora* gave many "spontaneous" hybrids even in the first year of his cultures of this species; (c) that hybrids of *B. rubella* were seen every year in his garden; (d) that many newly formed hybrids were found among his Swedish species after they had been growing for more than one year beside cultures of "southern species." There is no reason to suppose that successful crosses were any less frequent among the other elementary species in his cultures than they were between those elementary species and *B. grandiflora*, *B. rubella* or "other southern species." Since most of his elementary species undoubtedly belong in the species *B. bursa-pastoris* as here understood, they mostly breed together with full fertility, and the differentiating characters generally exhibiting the phenomena of dominance and recessiveness, the hybrids among these Swedish forms were as a rule indistinguishable from the self-fertilized parents, or differed so little as to be easily overlooked. The sterility of the hybrids produced when these forms are crossed with the species of the *rubella* group made it impossible to overlook them, thus giving apparent justification for the probably erroneous conclusion arrived at, that species of the *rubella* group are more prone to hybridize than are the elementary species of the *bursa-pastoris* group.

The fact that all crosses between representatives of the two groups of

species yield sterile hybrids so similar that they can scarcely be distinguished from each other, will explain the experience of Martrin-Donos, as cited by Focke (1881, p. 40), in finding *B. gracilis* (Grenier) in places where *B. rubella* does not occur. Since *any* species of the *rubella* group gives "*B. gracilis*" when crossed with any species of the *bursa-pastoris* group, it is probable that some other species of the *rubella* group was present but undetected in the region where Martrin-Donos found his *gracilis* specimens. On the basis of a similar observation, Almquist (1907, p. 88) concludes that hybrids, which do not involve any of the "southern species," are easily distinguishable because of their sterility. I have shown in the foregoing account that *B. penarthae*×*B. Heegeri* and *B. penarthae*×*B. bursa-pastoris* were largely sterile, and that crosses of *B. occidentalis* with *B. bursa-pastoris* or *B. Heegeri* are frequently sterile to a considerable degree, but this still leaves the fact unchanged that since most of the elementary species recognized by Almquist are included in the species *B. bursa-pastoris*, they will give no peculiarity of hybrid behavior which would make possible the recognition of their hybrids by mere inspection of any wild *Bursa* population in which they occur.

Summary

1. This is a progress report on a research program which seeks to articulate genetical studies with taxonomy, phytogeography, and cytology. Nearly 4000 pedigreed families have already yielded data from more than half a million individuals.

2. The genus *Bursa* (*Capsella*) is composed of a very large number of biotypes so related to one another that they can not be generally and consistently utilized as taxonomic units.

3. On the basis of genetical experiments ten forms have been recognized as species and two as subspecies. These forms are briefly characterized under the names: *Bursa bursa-pastoris* (L.) Britton, *B. bursa-pastoris apetala* (Opiz), *B. Heegeri* (Solms-Laubach), *B. occidentalis* n. sp., *B. occidentalis madeirae* n. subsp., *B. orientalis* n. sp., *B. djurdjurae* n. sp., *B. penarthae* n. sp., *B. grandiflora* (Bois.), *B. rubella* (Reut.), *B. Viguieri* (Blaringhem) and *B. tuscaloosae* n. sp.

4. These species and subspecies belong to two distinct groups with respect to their genetical behavior, the first eight forms here enumerated constituting the *bursa-pastoris* group, and the last four the *rubella* group.

5. Crosses between species within the same group yield more or less fertile F_1 hybrids.

6. Crosses between any species of one group and any species of the other group give sterile F_1 hybrids.

7. The fundamental distinction between these groups is one of chromosome number, the first eight forms, as here enumerated (*Bursa penarthae* not yet counted), having 16 haploid chromosomes, while the last four have 8 haploid chromosomes (Hill 1927).

8. *Hymenolobus procumbens* (L.) Nuttall (*Capsella procumbens* Fries), which has been frequently placed by taxonomists in the genus *Bursa*, is wholly disconnected genetically from the species here recognized as belonging to the latter genus, and it is rightly placed in another genus, as indicated by the adoption of Nuttall's name.

9. The segregations from the fertile hybrids demonstrate that the differences between these species are analyzable on the basis of Mendelian unit characters.

10. Factors for four characters in the *bursa-pastoris* group of species have been found to be usually or occasionally duplicated, each in two pairs of chromosomes, namely: (a) triangular form of capsules; (b) sinuses of the leaf-lobes reaching to the midrib; (c) non-coriaceous texture of the leaves; and (d) capacity to produce pollen.

11. No indications of duplicated factors have been found in the species of the *rubella* group.

12. Determination of genetic relationships by consideration of phenotypic resemblances may easily be in error owing to the fact that such resemblances are not always the result of genetic continuity. This is illustrated by the resemblance of the capsule form in *B. Heegeri* with that in the genus *Camelina*.

13. Because both *B. occidentalis* and *B. rubella* have concave-sided capsules, Almquist concluded that these two species are closely related, but the genetical experiments show that *B. occidentalis* is more closely related to *B. bursa-pastoris* and other species of the *bursa-pastoris* group than to *B. rubella*. It may conceivably have originated by a tetraploid mutation in *rubella*, while *B. bursa-pastoris* may bave originated by a similar mutation in some other species of the diploid (*rubella*) group.

14. Blaringhem's suggestion that *B. Viguieri* originated directly from *B. rubella* by a single mutation is shown to be untenable because these two species differ from each other by three major Mendelian factors and several minor ones.

15. My suggestions of ways in which duplication of the factor for triangular capsule conceivably might have taken place (Shull 1914, 1918) are shown to be inapplicable, because they were based on the tacit assumption that the chromosome number remained unchanged. It is now obvious that the duplication of factors resulted from the tetraploid mutation (or mutations) by which the *bursa-pastoris* group originated from the *rubella* group.

16. The occurrence of duplicated factors in the *bursa-pastoris* group proves that this group is a more recent derivative of the *rubella* group.

17. Two unexplained difficulties are mentioned: (a) that not all factors are duplicated in the tetraploid group; and (b) that presumably the original mutation resulted in the production of 8 groups of four homologous chromosomes (tetrasomes), but the genetical behavior indicates that these assort in definite pairs (disomes). How can the change from the tetrasomic to the double-disomic condition have taken place?

18. Notwithstanding the more recent origin of the *bursa-pastoris* group, it has a much wider geographical distribution than the *rubella* group.

19. It is considered a matter of fundamental significance that the increase in number of chromosomes in the *bursa-pastoris* group is correlated with greater variability, greater adaptability, greater vigor and greater hardiness.

20. The conclusion of Almquist that *B. grandiflora*, *B. rubella* and "other southern species" are more prone to hybridize than the Swedish species, is probably erroneous, and is explained by the fact that hybridizations between these southern species and the Swedish species produced sterile hybrids and were thus easily recognized, while hybridizations within the *bursa-pastoris* group, being usually fully fertile, have probably been mostly overlooked.

21. The fact that all the sterile hybrids between the two groups of species look so much alike that they could not be distinguished from eachother innature, readily explains the reported finding of *Bursa gracilis* (Gren.) in places where *B. rubella*, one of its supposed parents, does not occur.

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THE CONCEPT OF THE GENE[1]

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Nearly fifteen years ago[2] I attempted to defend the thesis that the Mendelian method of recording the facts of inheritance was simply a notation useful as a description of physiological facts. The argument was an elaboration of the proposition that the germ-cell unit of heredity, the gene, was an abstract, formless, characterless concept used for convenience in describing the results of breeding experiments. It was the ghost of an entity which might later be clothed with flesh, but its usefulness at the time was due to its adaptability to mathematical treatment. By postulating that the results derived from controlled matings were due to the activities of definite germ-cell units which could be manipulated arithmetically, investigators were able to formulate new experimental tests, and thus to open the way to further discovery; but these units could be given no intelligible interpretation in terms of geometry, chemistry, or physiology.

There is no reason today why the statements made at that time should be repudiated; rather should they be re-emphasized and made more rigorous, for, although numerous plants and animals have told us something of their heritage during the interim, and part of the phantasmagoria of the gene is now history, there is still a tendency to visualize it more concretely than is right and proper. And there is this further justification for reflecting on the groundwork of genetical theory from time to time; the difficulty which so many biologists experience in viewing biological phenomena with mathematical spectacles largely accounts for the limited diffusion of genetical philosophy outside of the ranks of specialists.

It is customary to regard biology as a science that moves slowly as compared with one of the supposedly more exact sciences such as chemistry. Probably this estimate is correct; yet the basic chemical concept, the modern atom, which is quite analogous to the genetic unit, remained a characterless mathematical notation for almost a century in spite of the alluring images drawn by Lord Kelvin and others. Although there had been atomic concepts in classical times, the modern atom was called into use by the discovery of the Law of Definite and Multiple Proportions, and served chemistry in a purely mathematical way until the discovery of radium. Since then, in a period practically coextensive with the history of genetics, it has been given such a quota of qualities by Bohr

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[1] Presented before the International Congress of Plant Sciences, Section of Genetics, Ithaca, New York, Aug. 19, 1926.

[2] East, E. M., The Mendelian notation as a description of physiological facts. Amer. Nat. **46:** 633–655. 1912.

and his co-workers that it can now take its place as a recognizably respectable citizen. During this same time-phase, the genetic unit unquestionably has reached nearly as high a social status.

A geneticist may make such a statement as this, confident that it is just; yet if he is not carried away by enthusiasm, he must also admit that this central concept, the theory of the gene, has not been incorporated into general biology as part of its legal estate. One should expect the period of probation usually required by science for new ideas, but in the case of the gene concept the time has been overly long. Yet it is not particularly strange that this should be so. From the time when Adam began to name the animals, as duly recorded in the second chapter of Genesis, down to the present, the greater part of all biological work has been historical and descriptive. Occasionally provisional causes for known facts have been adopted as a basis for further investigation, but the premises invariably have been simple and the conclusions direct. The evolution theory is a case in point. The supporting evidence is voluminous and its character varied, but the theory itself is free from intricacy or subtlety. The ideas involved in the term gene, on the other hand, are both complex and abstract. Because of the novelty of such conceptual notions, therefore, the biologist is inclined to approach them cautiously, sometimes even electing an attitude of arrogant distrust as a defense reaction.

The student of the living is not wholly to be blamed for his reactions. His position is not an easy one. The worker in the physical sciences, who has been making use of abstract concepts from the time of Hipparchus, has been somewhat inclined to patronize the biologist because the latter has occupied his mind with percepts or imagery familiar to the senses, to which the naïve investigator can cling with perfect faith in its reality. But the physicist is not nearly so tough-minded an individual as he professes to be. He is merely fortunate in being able to segregate his activities. He can do sound scientific work requiring the highest type of objective reasoning, and at the close of his working day lock up his professional personality in his laboratory, don a different ego with his dinner coat, and wander forth to dabble with theological dogma or to search for ectoplasm. The biologist has no such advantages. He is bound up with the problems of life at all times. He is psychiatrist to this lunatic asylum of the universe, and is familiar with all the crude absurdities with which man likes to deceive himself, including even the reason why the physicist likes to titillate his emotions in his idle hours. Thus it is difficult for him to find an emotional outlet of his own unless he abandons the proved tools with which he has successfully delved into the unknown and goes in wholly for entelechies or similar intentionally untestable figments of the imagination. The result is that his emotions and his intellect are forever embroiled. He hopes against hope that his old heritage of beliefs is true, that environment is all-powerful, that free-will prevails, that man is created in the image of God and is only a little lower than the angels. And he keeps on, a pathetic figure, proving that all the old folkways are myths, in spite of his desires and hopes. He does all this, but the conflict wearies him and makes him slow, slow to accept his own facts,

slow to push them to their logical end. I can see no other reason for the dozens of recent biologies, particularly genetic biologies, which have started bravely to build a scientific edifice to house man's beliefs, but have finished by decorating it with so many saintly old *mores* that it looked like a cathedral after all. I can see no other reason for the hesitation about accepting the theory of the gene. It is first the novelty of the thing, and second the effect it has on the old folkways.

In addition to the general difficulty experienced by biologists in accepting the new when derived from a more or less materialistic hypothesis, there is the specific embarrassment, which all of us experience to a greater or less degree, of distinguishing between the fiction postulated as an aid to gaining established fact, and the facts themselves. We come to believe in our fictions, and hesitate to give them up when they are no longer necessary. Science is almost as prone as theology to glorify its fictions into dogmas, though usually they do not stand in the way of progress for such lengths of time. Perhaps, therefore, it would be a service to genetics if the story of its building were called to mind every little while, that we may actually see what has been useful as scaffolding and what remains as structure.

Mendel's postulate of a germ-cell unit, which we may call the gene in order not to change the terminology, was not a novelty. Quantitative science requires units. They have been used from time immemorial. As soon as biologists began to speculate on the possibility of reducing the phenomena of heredity to law, therefore, units of description were proposed. At about the same time appeared the suggestions of Nägeli, Spencer, Darwin, Weismann and Mendel. *Mendel made his units useful.*

It appeared that the pea experiments could be interpreted by units from a duplex organization consisting of homologous pairs in the somatic cells, which could undergo varied associations with other units without being modified and afterwards appear in the germ cells in simplex organization produced by the permutations and combinations of one member of each homologous pair. Mendel's generalizations were three in number, the segregation of homologous factors without change of identity in the formation of the germ cells, the recombination of the products of this segregation in all possible combinations, and the formation of zygotes by random matings among the gametes thus produced.

These three laws have now been shown to be special cases of more general phenomena. The law of segregation retains the original idea of concrete units undisturbed in their own identity by association with other units in the germplasm; but the idea of a double set of hereditary factors, serially homologous, becoming two separate sets by the required choice of *one* of each pair of factor mates, no longer tells the whole story. The chromatin has been proved to be the gene carrier; its distribution gives us the distribution of the genes. And, like the activities of human beings, the behavior of chromatin is not always according to the regular or accepted mode.

Nor is the second law of Mendel, chance recombination of genes, a complete expression of the facts. Generally speaking, the individual chromosomes behave as if they were more or less independent transportation systems for packets of genes; but these packets may be broken up and interchanged according to regular rules, the rules themselves being subject to the influence both of external conditions and of hereditary factors. It is even possible that several chromosomes, ordinarily segregating independently, may tend to keep their maternal and paternal associations. At least one may so interpret some recent work of Gates[3] on mice where a series of dominant characters in the house mouse which ordinarily assort independently showed a tendency to linkage when opposed to recessive characters in the Japanese waltzer.

Finally, it would not be at all strange if the random mating of gametes to form zygotes is also a special case. Clearly random mating is what usually occurs; but so many cases are now known where there is a differential *opportunity* for gametic unions that it is quite probable that it will soon be impossible to draw a sharp line between equal and unequal opportunities for fertilization.

These cases where the original Mendelian Laws have broken down have been of paramount importance to genetics. Progress is nearly always due to the analysis of exceptional phenomena. A complete list would practically be the history of the science.

The interaction of two independently inherited factors to bring about a single visible effect resulted in an early extension of theory. A series of dominants, each one epistatic to the next lower, called forth the Presence and Absence Theory. Probably no geneticist ever believed in quite such a crude Presence and Absence Theory as Morgan has made out; but that is of little consequence. The theory served a purpose and gave way to a clearer one, after analysis of exceptional cases had shown that an individual heterozygous for a factor is different from the individual that is haploid for it, and that in general both dominants and recessives function actively, though either may be inactivated by the presence of other genes and possibly by changes in external conditions.

Again, these "irregular" cases have told us much about the relation of genes to each other. No one now attributes a single specific effect to one gene or interprets a particular organic character as the result of one gene's activity. Single genes affect many characters; each character is built up by the action of many genes. These facts have been proved most beautifully by Bridges' work on sex in *Drosophila melanogaster*.

Perhaps the greatest, or at least the most spectacular, result of modern pedigree culture analysis supplemented by cytological investigations, however, is the contribution to our knowledge of the architecture of the germ-cells. Proof of the linear arrangement of the genes in the chromosomes, which was clinched by non-disjunction and reduplication phenomena, was an astounding achievement of inductive experiment. The basis of all these discoveries is "crossover"

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[3] Gates, W. H. The Japanese waltzing mouse: its origin, heredity, and relation to the genetic characters of other varieties. Pub. Carn. Inst. Wash. No. 337, 1926.

frequency, or breaks in linkage. Let us discuss this genetic tool. It is perhaps more worth while than the discussion of any other genetic tools or attainments, since a little meditation brings out clearly that we have after all reached only a relative truth. The gene even now has no very concrete meaning. It is a slowly shifting, imaginative figure, clothed indeed with some known qualities, but still vague, very vague.

Take first the question as to whether a given effect is due to two genes or to one gene. A plant, let us say, has a red flower and a red fruit. Varieties exist with white flowers and white fruits. Ordinarily, by crossing, one can find out shortly whether the effect comes from one gene or from two, and if from two genes, whether they lie in separate chromosomes. But the matter is largely a question of frequency. If the cross is $AB \times ab$ and there is a five per cent crossover, sufficient *Ab* and *aB* gametes can be traced to satisfy our doubts. But suppose the crossover is one one hundredth of one per cent. Is it a crossover? or is it a mutation? If both *Ab* and *aB* gametes can be traced we call it a crossover; if only *Ab* or *aB* gametes can be traced, which is quite likely to be the case—especially when investigating megasporogenesis—we should call it a mutation.

Multiple allelomorphism is another illustration of the artificiality of the system. When a change occurs, which when crossed back with the original stock gives only the two classes, we say a mutation has taken place. A third, a fourth, any number of changes occur in this character. When any two are crossed and only these two recovered in the F_2 generation, we say that a series of multiple allelomorphs has been established. Such usage is logical and desirable, but it by no means proves that chemical rearrangement has occurred in each case at one definite place in the substance of the chromosome. Morgan[4] believes that it does, but his point is not well taken. He says:

"It might be claimed that the phenomenon of multiple allelomorphism is an expression, not of changes in the same locus, but changes in neighboring loci that are so close together that crossing-over never occurs between them. Suppose, by way of example, a mutation took place near the white locus that gave the eye color eosin. If the mutation occurred in a chromosome that had already a white gene, then the new eye color would be due to the combined action of white and eosin. If eosin arose in this way, then, when such an eosin fly is crossed to the original white stock it should give white, since the effect of the new recessive eosin in one chromosome of the F_1 is cancelled by the effect of the normal allelomorph of eosin. In fact, the combination gives an eye color that is not white but is intermediate between white and eosin. If, on the other hand, the mutation to eosin occurred in an X-chromosome not having a white gene, but its normal wild-type allelomorph, then, whenever an eosin fly is crossed to a white fly the female offspring should have red eyes, since each X carries the normal allelomorph of the recessive gene (white or eosin) of the other X. But

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[4] Morgan, T. H., Bridges, C. B., and Sturtevant, A. H., The genetics of Drosophila. Bibliographica Genetica II, 1925. 262 p. (See p. 37.)

is fact, an eye-color not red but intermediate between white and eosin results, demonstrating again that eosin and white are not closely linked recessive mutants. The same arguments apply to each of the other nine members of the same series. In the light of these facts it is surprising that statements are still made that the evidence of multiple mutation in the same locus is not established."

I do not believe that this argument is cogent. There have been twelve mutations at the so-called "white locus" in *Drosophila melanogaster*. Let us assume that a change has taken place in each case at a different "spot" in a linear series of twelve "spots" all so close together, or for some reason bound so strongly together, that crossing-over never takes place. A change takes place at No. 1 link which produces white, a change takes place at No. 2 link which produces pink, a change takes place at No. 3 link which produces eosin. Now Morgan says that "if eosin arose in this way, then, when such an eosin fly is crossed to the original white stock it should give white, since the effect of the new recessive eosin in one chromosome of the F_1 is canceled by the effect of the normal allelomorph of eosin." This is either imagining too much or not enough. The change in the No. 1 link gave white without respect to what remained, the change in No. 3 link gave eosin without respect to what remained. A cross between the two would give whatever the combined effects of the haploid white and the haploid eosin happened to be, and only these two forms would be recovered in the F_2 generation, since no crossing-over has been postulated.

Such a question holds little interest for theoretical genetics at present, but it does bear on the nature of the gene. Morgan has endeavored to make a determination of gene size at a given period in gametogenesis. He has done this by calculating the volume of the chromosomes at this phase and then taking as the unit size that portion of the chromosome giving the smallest crossover value. There is difficulty in making both calculations, particularly the second; but by taking approximately the lowest value of the crossover curve, he arrives at a value which is probably of a good accuracy. The diameter of the gene, thus calculated, turns out to be [illegible] of a micron. The number of genes he estimates at 2000.

The gene, when estimated in this manner, proves to be but little larger than the calculated size (also an interpretation, of course) of the molecule of haemoglobin. It is an ingenious and valuable computation, but if we are not careful it may lead our ideas astray. The estimate of the total number of genes is based on the assumption that all genes are alike, that our unit is a special case instead of a genetic unit. The *Drosophila* chromosome "map" contains vacant spaces. Genes are more frequent in some parts than in others. It is assumed from this fact that mutations have occurred—or have been discovered—more frequently in some parts of the chromosome than in others. But it is also reasonable to suppose that genes vary in size. Separate genes may not have been discovered because non-crossover space is large in some regions and small in other regions.

Now such speculation is useless unless it serves to aid in holding our minds open for future possibilities. At present one might a classification of the gene

is not wise because of its effect on the reception of data concerning the frequency of mutation. It is very fortunate that the gene as we have thus far clothed it with flesh has a sufficiently high degree of stability to be extensively useful. Naturally the ultimate unit of any nomenclature must be stable, but sometimes it is necessary to utilize several units to serve all purposes. Synthetic and analytic chemistry finds the molecule useful as a unit, though molecules differ greatly in size and in stability. For other purposes the atom is valuable. In final analysis, however, recourse has been made to a still less variable unit, the electron. Is genetics going to find it necessary to deal with a lesser unit of heredity in order to gain stability? This question is difficult to answer. Eyster's work deals with an extremely variable gene. It may be necessary to postulate a new unit to deal with it or with similar phenomena effectively. But perhaps, with the analogy of chemistry before us, we can make the old conception serve, if we keep in mind that we know as yet neither the upper nor the lower limit for gene size, neither the upper nor the lower limit for gene stability, and that we have drawn our pictures merely by the aid of crossover values. It is reasonable to suppose that genes are subject to the laws of chemistry. Let us assume that they are organized like molecules. It would follow that some gene-molecules are enormous as compared with others, that some are highly stable and others relatively unstable; and it might even follow that there is a correlation between size and instability. Furthermore, since chemical molecules can undergo various reorganizations without loss of substance, perhaps gene-molecules can do the same. These reorganizations may or may not be equally possible in both directions: that is to say, each state may not have the same coefficient of stability. Again, on chemical analogy, only a limited number of reorganizations should fit the communistic purposes of the organism; the majority of mutations should be lethal. And finally, since increasing molecular complexity decreases the available directions of change, though it increases the number of possible permutations, orthogenesis is understandable. This last sentence is merely a statement of the fact that although highly organized molecules, of the aromatic series or of the aliphatic series, let us say, have many possibilities of easy reorganization, these reactions are more limited in direction because of the large and stable molecular nucleus than are of those of certain simpler compounds.

We arrive, therefore, at the same port from which we departed when our discussion began. The genes are units useful in concise descriptions of the phenomena of heredity. Their place of residence is the chromosomes. Their behavior brings about the observed facts of genetics. For the rest, what we know about them is merely an interpretation of crossover frequency. In terms of geometry, chemistry, physics, or mechanics, we can give them no description whatever.

THE GENE AS THE BASIS OF LIFE[1]

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1. The Localization of Genes

What is meant in this paper by the term "gene" material is any substance which, in given surroundings— protoplasmic or otherwise—is capable of causing the reproduction of its own specific composition, but which can nevertheless change repeatedly—"mutate"—and yet retain the property of reproducing itself in its various new forms. There is clear evidence that such material is to be found in the chromatin (where it is linearly arranged), and to some extent in the chloroplastid primordia and their derivatives, and there is no reason to believe that it exists anywhere else within the cell. In this connection it may also be noted that in the most primitive organisms which contain a chlorophyll-like substance the chromosomes do not seem yet to have become distinctly grouped apart from their cytoplasmic surroundings, into a walled-off nucleus; hence the genes more directly associated with the chlorophyll, on the one hand, and the nuclear genes, on the other hand, may well have had a common origin, and may only in later phylogeny have become separated.

2. The Size and Number of Genes

In order first to make our topic as concrete as possible let us now put to ourselves the question: *What is the size of the chromosomal gene*,—or, if a more pedantic formulation be demanded, what is the size of the smallest portion of protoplasm, separable under some conditions by chromosome interchange, which includes all the substance *specifically* necessary for the transmission of a single given Mendelian *difference?* One way to approach this question is through an attempted determination of the number of genes (taken in this sense), since this number may then be divided into the known bulk of the chromatin containing these genes. The present author, after an attack upon this problem, stated (in a paper written in collaboration with Altenburg in 1919) that the minimum number of genes in the X chromosomes of *Drosophila* must be about 500. (The total haploid number would consequently be about 2000.) From this it readily followed (as I stated in 1921) that the gene is an "ultra-microscopic particle"—though the determination did not yet seem precise enough to warrant an overt comparison with the size of protein molecules, or of other, less familiar, colloidal bodies of the cell, and such comparison was therefore left to the reader's

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[1] Presented before the International Congress of Plant Sciences, Section of Genetics, Symposium on "The Gene", Ithaca, New York, Aug. 19, 1926.

imagination.[2] As I have never explained the method by which these conclusions were arrived at this may be briefly sketched here, together with some recent refinements of it, and the results thereby obtained.

a. THE EVIDENCE FROM MUTATIONS IN IDENTICAL LOCI

We cannot know specifically of the existence of any genes except those in which, by the chance occurrence of mutation, a Mendelizing difference has arisen, and so we cannot directly count the total number of genes. However, we can estimate their number from the number of those that have mutated once, twice, three times, etc. The problem here is the same as if we had a bag containing a large but finite number of balls, whose number we wished to know. We may suppose that every ball is differently marked from every other ball, and that we are allowed to reach into the bag a definite number of times, blindly pull out a ball each time, then note its mark, and replace it at random before drawing again. The larger the total number of balls in the bag the less often would we tend to draw the same ball over again, and by noting how often we obtained identical draws, in comparison with unique draws, we could, by the aid of certain principles derived from the mathematics of probability, calculate the approximate total number of balls that the bag must contain.

In the case of random draws—which would correspond to the condition that all loci in the chromosome mutate equally readily (to forms equally detectable)—the mathematics is simple, for then there is the same ratio between the number of balls drawn at least once (n_1) and the total number of balls in the bag (n_0) as between the number drawn at least twice (n_2) and the number drawn at least once, that is, $\frac{n_1}{n_0}=\frac{n_2}{n_1}$. Genetically, n_0 would represent the total number of gene-loci, n_1 the number in which any mutation at all had been observed, and n_2 the number in which 2 or more mutations had been observed. The latter ratio, $\frac{n_2}{n_1}$, in turn, is the same as the ratio of the number drawn at least 3 times to the number drawn at least twice, that is, $\frac{n_2}{n_1}=\frac{n_3}{n_2}$, and this same relation holds throughout, that is,

$$\frac{n_{x+1}}{n_x}=r \text{ (a constant)}, \qquad \text{(Formula 1)}$$

within the limits of error due to random sampling. On account of these relations n_0, the total number, can in such a case be calculated from any of the formulae:

$$n_0=\frac{n_1}{r}, \text{ or } \frac{n_2}{r^2}, \text{ or } \frac{n_x}{r^x}, \qquad \text{(Formula 2)}$$

or, substituting values of n for r, $n_0=\frac{n_1^2}{n_2}$, or $\frac{n_2^3}{n_3^2}$, or

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[2] The estimate of number or size thus made has since been used in a publication by Sturtevant (1921), and later by others (e.g., Morgan, 1922, Morgan, Bridges, and Sturtevant, 1925).

$$n_0 = \frac{n_x^{x+1}}{n_{x+1}^{x}} \qquad \text{(Formula 2a)}$$

In our genetic case, however, we do not have strictly "random draws," as some loci (like that containing the normal allelomorph of white eye) obviously mutate, in a detectable way, much oftener than do others (for example, the locus of the normal allelomorph of "dichaete"); this is as if some balls were more apt to escape our fingers than were others. In consequence, we obtain more draws of a higher recurrence-number, in proportion to those of lower recurrence-number, than given by the ratio of the latter to draws of still lower recurrence-number, that is, $\frac{n_{x+2}}{n_{x+1}} > \frac{n_{x+1}}{n_x}$; the unique draws thus form a smaller proportion of the entire number of balls, or loci, than calculated, on the basis of formula 2, from the data on recurrence. Hence the total number of balls, or genes, so arrived at, falls short of the actual number, and we obtain only a *minimum* estimate of the total number of genes. This gives us, correspondingly, a *maximum* estimate, certainly much too large, of the average size of the individual gene.

The above proposition is capable of rigorous proof. Starting with the condition of equal mutability, we have in a given group of genes, $n_{o\cdot a}$ in number, by formula (2), $n_{o\cdot a} = n_{1\cdot a}^2 / n_{2\cdot a}$. Now suppose that there is another group of genes, also alike in their mutation rate, but having a different rate from the first group; these genes are $n_{o\cdot b}$ in number, and for them $n_{o\cdot b} = n_{1\cdot b}^2 / n_{2\cdot b}$. If then the two groups were mixed, the total number ($n_{o\cdot a+b}$) would be $n_{o\cdot a} + n_{o\cdot b}$; the number found to mutate at least once ($n_{1\cdot a+b}$) would be $n_{1\cdot a} + n_{1\cdot b}$; those mutating at least twice (n_{2a+b}) would be $n_{2\cdot a} + n_{2\cdot b}$; etc. If we applied formula (2) to the lumped data obtained from the mixed group we would write:

$$n_{0\cdot a+b} = \frac{(n_{1\cdot a+b})^2}{n_{2\cdot a+b}},$$

which would be the same as saying:

$$(n_{0\cdot a} + n_{0\cdot b}) = \frac{(n_{1\cdot a} + n_{1\cdot b})^2}{n_{2\cdot a} + n_{2\cdot b}},$$

whereas actually

$$(n_{0\cdot a} + n_{0\cdot b}) = \frac{n_1^2{}_{\cdot a}}{n_{2\cdot a}} + \frac{n_1^2{}_{\cdot b}}{n_{2\cdot b}}.$$

It can be proved that the latter, true expression is necessarily a larger quantity than the former, assumed expression. To prove this, i.e., that

$$\frac{n_1^2{}_{\cdot a}}{n_{2\cdot a}} + \frac{n_1^2{}_{\cdot b}}{n_{2\cdot b}} > \frac{(n_{1\cdot a} + n_1^2{}_{\cdot b})}{n_{2\cdot a} + n_{2\cdot b}};$$

reduce all terms to the common denominator $n_{2\cdot a} \cdot n_{2\cdot b} \cdot (n_{2\cdot a} + n_{2\cdot b})$; the numerators then become $n_{1\cdot a}^2 \cdot n_{2\cdot a} \cdot n_{2\cdot b} + n_{1\cdot a}^2 \cdot n_{2\cdot b}^2 + n_{1\cdot b}^2 \cdot n_{2\cdot a}^2 + n_{1\cdot b}^2 \cdot n_{2\cdot a} \cdot n_{2\cdot b} > n_{1\cdot a}^2 \cdot n_{2\cdot a} \cdot n_{2\cdot b} + 2n_{1\cdot a} \cdot n_{1\cdot b} \cdot n_{2\cdot a} \cdot n_{2\cdot b} + n_{1\cdot b}^2 \cdot n_{2\cdot a} \cdot n_{2\cdot b}$. Transposing terms and adding: $n_{1\cdot a}^2 \cdot n_{2\cdot b}^2 - 2n_{1\cdot a} \cdot n_{1\cdot b} \cdot n_{2\cdot a} \cdot n_{2\cdot b} + n_{1\cdot b}^2 \cdot n_{2\cdot a}^2 > O$; this reduces to $(n_{1\cdot a} \cdot n_{2\cdot b} - n_{1\cdot b} \cdot n_{2\cdot a})^2 > O$, which is necessarily true, owing to the fact that the square of any real number is positive. This means that the actual total number of genes ($n_{o\cdot a+b}$), which is equal to

$$\left(\frac{n_1^2{}_{\cdot a}}{n_{2\cdot a}} + \frac{n_1^2{}_{\cdot b}}{n_{2\cdot b}}\right),$$

is larger than that calculated by taking $n_{o \cdot a+b} = (n_{1 \cdot a+b})^2 / n_{2 \cdot a+b}$, which represents the application of formula (2) to the lumped data on the two sets of genes.

This result for two sets of genes can now be generalized by showing that, no matter how many sets of genes of different mutation rates are lumped together, if the value of the total number, $n_{o \cdot a+b+c+ \cdots i}$, is larger than that represented (according to formula 2) by the expression

$$\frac{(n_{1 \cdot a+b+c \cdots i})^2}{n_{2 \cdot a+b+c+ \cdots i}},$$

then, if we add to the lot still another set of genes ($n_{o \cdot k}$), the value of the new total number $n_{o \cdot a+b+c+ \cdots i+k}$, is larger than that given (according to formula 2) by the expression

$$\frac{(n_{1 \cdot a+b+c+ \cdots i+k})^2}{n_{2 \cdot a+b+c+ \cdots i+k}}.$$

For

$$n_{0 \cdot a+b+c+ \cdots i+k} = n_{0 \cdot a+b+c+ \cdots i} + n_{0 \cdot k} = n_{0 \cdot a+b+c+ \cdots i} + \frac{(n_{1 \cdot k})^2}{n_{2 \cdot k}} > \frac{(n_{1 \cdot a+b+c+ \cdots i})^2}{n_{2 \cdot a+b+c+ \cdots i}} + \frac{(n_{1 \cdot k})^2}{n_{2 \cdot k}}$$

$\left(\text{by our original assumption that } n_{0 \cdot a+b+c+ \cdots i} > \frac{(n_{1 \cdot a+b+c+ \cdots i})^2}{n_{2 \cdot a+b+c+ \cdots i}}\right)$. But

$$\frac{(n_{1 \cdot a+b+c+ \cdots i})^2}{n_{a \cdot 2+b+c+ \cdots i}} + \frac{(n_{1 \cdot k})^2}{n_{2 \cdot k}} > \frac{(n_{1 \cdot a+b+c+ \cdots i} + n_{1 \cdot k})^2}{n_{2 \cdot a+b+c+ \cdots i} + n_{2 \cdot k}}$$

(by the same proof as given in the preceding paragraph, changing only the subscripts of n). Hence,

$$n_{0 \cdot a+b+c+ \cdots i+k} > \frac{(n_{1 \cdot a+b+c+ \cdots i+k})^2}{n_{2 \cdot a+b+c+ \cdots i+k}}.$$

As this relation has previously been proved to be valid for a lot consisting of two sets ($n_{o \cdot a}$ and $n_{o \cdot b}$), and since it is here proved that the addition of an extra set never changes its validity, provided it was true in the previous case (before the addition), it follows, according to the principle of mathematical induction, that the relation is true in general.

We can allow, to some extent, for the above source of error, when we have sufficient figures to give us an idea of the relation, not only of the number of unique draws to double draws, but of draws of higher frequencies of recurrence to each other. To illustrate this procedure, let us take the latest figures giving the number of recurrences in the loci that have been studied in *Drosophila melanogaster* (compiled chiefly by Bridges, and given in Bibliographia Genetica II), and use in connection with this the approximate number of separate loci in which any mutations at all (including "unique" mutations) are known to have occurred—excluding, however, those in which, for any reason, possible recurrences could scarcely have been recognized as such, in case they had taken place. We then obtain the figures in table 1 (see column "a+b," figures not in brackets):

Now we can, if we choose, use the figures giving the higher numbers of recurrences to estimate approximately the total number of genes having a high mutation rate (r_b), on the basis of our simple principle that, for genes of a given mutation rate, the ratio (r) of each number (n_x) to the one next below it in recurrence-frequency (n_{x-1}) is the same as the ratio of the latter to the number similarly below itself. The ratios in the case of values of n ranging from $n_{8.a+b}$ to $n_{4.a+b}$ are found to approximate 0.77. Since we may take the values of n_b as practically equal to those of n_{a+b} in the case of these high recurrence-fre-

quencies, we may put $r_b = 0.77$ and $n_{4.b} = 23$; from these values we may then obtain the simple geometric series for $n_{3.b}$ to $n_{0.b}$ which is given in brackets in column "b." The figures $n_{3.b}$, $n_{2.b}$ and $n_{1.b}$ so obtained may then be subtracted from the observed, "lumped" figures for 3, 2, and 1 occurrences ($n_{3.a+b}$, $n_{2.a+b}$, and $n_{1.a+b}$), to get the calculated figures of column "a," showing the numbers of loci of lower mutation rate (r_a) having 3, 2, or 1 occurrences. From the ratios of the numbers $n_{3.a}$, $n_{2.a}$ and $n_{1.a}$ to each other we next find that $r_a = 0.105$ in these

TABLE 1. SHOWING NUMBERS OF LOCI IN DROSOPHILA MELANOGASTER HAVING VARIOUS FREQUENCIES OF OBSERVED OCCURRENCE OF MUTATION.
(ALL CALCULATED NUMBERS OF LOCI ARE GIVEN IN BRACKETS.)

Number of times mutation has been observed in same locus	Total numbers ("a+b") of loci (of all mutation rates) having stated recurrence frequency	Designations of numbers (in a+b column)	Numbers ("b") of loci of higher mutation rates [average rate = r_b = .77]	Designations of numbers in (b) column	Numbers ("a") of loci of lower mutation rates [average rate = r_a = .105]	Designations of numbers in (a) column
8 or more times	9	$n_{8 \cdot a+b}$	9	$n_{8 \cdot b}$		$n_{8 \cdot a}$
7 " " "	11	$n_{7 \cdot a+b}$	11	$n_{7 \cdot b}$		$n_{7 \cdot a}$
6 " " "	16	$n_{6 \cdot a+b}$	16	$n_{6 \cdot b}$		$n_{6 \cdot a}$
5 " " "	19	$n_{5 \cdot a+b}$	19	$n_{5 \cdot b}$		$n_{5 \cdot a}$
4 " " "	23	$n_{4 \cdot a+b}$	23	$n_{4 \cdot b}$		$n_{4 \cdot a}$
3 " " "	31	$n_{3 \cdot a+b}$	[30]	$n_{3 \cdot b}$	[1]	$n_{3 \cdot a}$
2 " " "	51	$n_{2 \cdot a+b}$	[39]	$n_{2 \cdot b}$	[12]	$n_{2 \cdot a}$
1 " " "	165	$n_{1 \cdot a+b}$	[50.6]	$n_{1 \cdot b}$	[114.4]	$n_{1 \cdot a}$
0 " " "	[1152 Calculated from $n_{0 \cdot a} + n_{0 \cdot b}$]	$n_{0 \cdot a+b}$	[61]	$n_{0 \cdot b}$	[1091]	$n_{0 \cdot a}$

experiments. This, multiplied by the value of $n_{1.a}$, gives $n_{0.a}$, the total number of genes of mutation rate r_a, as 1091. The addition of $n_{0.a}$ and $n_{0.b}$ finally gives us an estimated grand total of genes of "both" mutation rates.

It will be seen that the figure gotten by this rather crude approximation process is 1152. This is still a low minimum estimate, since it only divides the genes into two categories of mutability, whereas the genes in each of these categories really differ very much among themselves; hence the estimated number for each category is too small. For greater accuracy more categories could be used, or a more elaborate method could be employed as follows:

Draw a hypothetical curve of "mutability frequency," in which the abscissae represent r, the mutation rates, and the ordinates n_0, the numbers of loci having such mutation rates. The term "mutation rate" here has a similar meaning to the ratio "r" previously used; it may be defined as the "chance"

that at least one mutation would have been observed in the locus under consideration, throughout the entire series of experiments which have been carried out on the organism in question. The actual shape of this curve is of course unknown, but its area, N (or "$n_{0.\Sigma x}$"), represents in any event the total number of genes, of all mutation rates combined, and it is this number which we desire to obtain. Now in the case of the $n_{0.a}$ genes having a given mutation rate, r_a, we would, by our definition of r, have observed $r_a\, n_{0.a}$ genes in which at least one mutation had occurred, and, by formula (1), which applies properly to genes of a given mutation rate, we have $r_a(r_a n_{0.a})$ of these genes, or $r_a^2 n_{0.a}$, in which at least two mutations have been observed, $r_a^3 n_{0.a}$ in which at least three were found, and so forth ($r_a^x n_{0.a}$ in which x were found). The same rule applies for each value of r. Hence the sum of $r\, n_0$ taken for each value of r (i.e. $\sum r\, n_0$) is the total number of loci in which at least 1 mutation has been observed, in the lumped data; $\sum r^2 n_0$ is the total number of loci in which at least 2 mutations have been observed, and so forth ($\sum r^x n_0$ being the number in which at least x mutations have been observed). Our data derived from the experimental work give us the numerical values of these expressions. But $\sum r\, n_0$ is what is known in mathematics as the "first moment" of the curve involving r and n_0, taken not from its mean but from its origin on the left hand, and multiplied by N, the area; this would ordinarily be represented by the symbols $\bar{\mu}_1 N$; likewise $\sum r^2 n_0$ is $\bar{\mu}_2 N$, $\sum r^3 n_0$ is $\bar{\mu}_3 N$, etc. ($\sum r^x n_0$ being $\bar{\mu}_x N$). The moments of a curve, even those taken from its origin rather than from its mean, can usually be used to reconstruct the curve and to obtain its area, N, but in this case the problem is complicated by our knowing only the product of these moments times N itself. To reach an approximate solution in such a case we can proceed by first assuming various apparently possible values of N, and, on each given assumption, finding $\bar{\mu}_1$, $\bar{\mu}_2$, $\bar{\mu}_3$, etc. (by dividing N into the obtained figures for $\bar{\mu}_1 N$, $\bar{\mu}_2 N$, $\bar{\mu}_3 N$, etc.). Using the hypothetical values of the moments so obtained (for each assumed value of N) we may then follow the well-known biometrical rules for obtaining, from its moments, the approximate equation of the curve involving r and n_0. It may next be integrated to find its area, N, and a comparison may be made between the N thus gotten and the N originally assumed. That assumed value of N which results in a final value agreeing best with the originally assumed one may be taken as nearest the correct value of N, the total number of genes, of all mutation rates. We would scarcely be justified in applying this very cumbersome method to our present meager data, as the results could not differ significantly from those more simply arrived at.

It should be emphasized that, in the above method and also in the previous rougher approximation method, r, the "mutation rate," was really the rate of *detectable* mutations, that is, the frequency with which, in an indefinite series of "experiments" similar to that already performed (regarding, for example, all the *Drosophila* work to date as "one experiment" from this point of view), mutations in the loci in question would have been *discovered*. Now, within a given "experiment" (in this extended sense) the detectability of a given sort of mutation may vary greatly, owing to differences both in physical, in genetic,

and in psychological factors, from cross to cross, from observer to observer, and in the course of practice; the actual time-rate of mutation itself, in given loci, may conceivably change also. However, no such variations in the distribution of detectable mutations, due to any of these causes, can in any way invalidate the above methods, as the latter do not deal with the temporal or spatial distributions of the mutations occurring within an "experiment," but only with their total chances of occurrence (in given loci) for the "experiment" as a whole.

There is, however, a real source of difficulty in applying the methods previously outlined. That is, it is likely that data like the above will never allow us to ascertain at all closely the shape of the curve of mutability-frequency near its left-hand extreme, where the genes of very low mutation rate are represented. The latter genes might be very numerous even though our data (which tend to comprise mostly genes of higher mutation rate) gave us very little indication of their existence, and still less of how their numbers varied as the mutation rate considered became progressively lower. In that event, all we could do would be to determine the minimum of genes (the smallest N) that yielded a curve consistent with the data.

One obvious reason why calculations based on data like the above will probably leave the number of genes having a very low rate of detectable mutation so indeterminably large is because in such work we have been detecting little else than the mutations in epidermal morphology and pigmentation. Over 90 per cent of the mutations numbered in the table involve eye, wing, or bristle formation, or coloration of eye or skin. Most mutations causing physiological or even internal morphological changes may well escape our observation. For it is evident from the data on recurrence of mutation in particular loci that a given locus usually mutates preferentially in a certain direction; if, then, we are detecting mutations only in some other direction, most of the genes that mutate chiefly in the first direction may be lost to our view. In illustration of this, we may note that very few of the numerous loci known to affect eye color in *Drosophila* have been found also (on mutation) to cause changes in wing shape, or, in fact, in any of the other commonly noted characters, and so if some condition had prevented us from noting the eye color changes hardly any of these loci would have been discovered, certainly not enough to render any data on recurrences of mutation among them significant. Their rate of mutation (that is, of detectable mutation) would have been practically subliminal. The range of detectable character changes does, to be sure, tend to widen, as our technique of detection improves, yet it may always remain too limited to allow significant data to be obtained, in the course of ordinary genetic work, on the mutations of the majority of loci.

The above obstacle to the obtaining of any but an unknowably low minimum estimate of the number of genes can perhaps be largely avoided if we make use of lethal rather than of "visible" mutations in our calculations. For it seems likely that most genes, when the change in them becomes extreme, may produce lethal effects. Certainly a very large proportion of the ordinary "visible"

mutations of all kinds, in *Drosophila,* carry in their train some reduction of viability; in the case of multiple allelomorph series the more extreme allelomorphs (visibly differing most from the normal) commonly cause the greatest reduction in viability; and, despite the small amount of investigation of this matter yet carried out, a number of cases are already known in which very extreme allelomorphs of ordinary, non-lethal "visible" mutant genes are themselves completely lethal when homozygous. In studies of lethal mutation rates, then, would seem to lie the road leading nearer to a solution of our problem of the number of genes. It is, however, necessary in such work with lethals to save stocks of each lethal, and to make innumerable crosses for the determination of allelomorphism. Only autosomal lethals can be used for such determinations, since of course males with sex-linked lethals are not available for crossing. Hence an elaborate and costly campaign will be needed, and it must be a long time before sufficient data on the recurrence of lethals can be accumulated to render possible a more adequate attack on the question at issue. The data so far obtained by the present writer, in experiments with lethal mutation rate, would, if taken at their "face value," yield a figure for gene number approximately like that obtained above—in fact, slightly smaller—but the probable error of the number of recurrences, in such a small total, is far too large, as yet, to make even the order of magnitude of the result significant.

b. EVIDENCE FROM CROSSOVER FREQUENCIES

A more obvious method than the above, for trying to count the genes in *Drosophila,* is to multiply the total number of so-called "units" in the linkage maps (which is about 285) by the number of genes which may occur in one unit. This leads us to the figure of 285×5, or 1425 genes, if we accept one fifth of a unit as the shortest usual "distance," in terms of percentage of separations, between genes—i.e., 5 genes to a section giving 1 per cent of crossing over. There are several well-determined cases of approximately this degree of association, in regions of the map where the genes do not seem, otherwise, to be closely crowded, and where, consequently, it is not suspected that the unusual strength of linkage between the two loci in question may be due merely to local hindrance to crossing over rather than really to close proximity. The best of these cases is perhaps Claussen's case of 0.2 per cent jaunty-black crossing over in chromosome II (though this is not very far from the crowded central region); another is Bridges' case of tan and lozenge in chromosome I. Other cases, of still closer linkage, are as yet open to the above or other suspicions.

In the above calculations based on the strength of linkage between near-by genes in ordinary chromosome regions, it would have been more correct to have taken account of the fact just alluded to: that certain chromosome regions (especially some central and some terminal ones) probably comprise longer regions than their map values would indicate,—as shown by the clustering of the genes there, which is almost certainly caused by a relatively low crossover frequency. The real lengths of these regions, in proportion to the "units" of the other regions, may be approximately gauged by the relative numbers of

mutated loci therein observed. If we make this allowance (assuming at the same time that there are not converse regions, of especially low crossover frequency), we increase the estimated total length of the chromatin, and consequently the estimated total number of genes, by at least one fourth again. This would make the estimated number of genes about 1800.

May we, however, conclude that just because genes have not been found "closer" together than one fifth of a unit they are not in fact closer together? How may we determine whether our sample of genes, obtained by their "chance" mutation, gives us a fair estimate of the closest distance between the genes? This question too is soluble by mathematical means. Suppose we are dealing with "n" genes, in a map "m" units long, and wish to find the chance that, given a random distribution of these genes, *none* of them should happen to fall within a given distance, "d," of each other, where d represents some fraction of a unit. The first of the n genes considered may lie in any position along the given map, but the second gene, in order *not* to be within the distance d of the first, must not fall in either of the spaces, d in length, lying to the right or to the left of the first gene, being thus excluded from a total length $= 2d$, of the entire map, m. The chance of its thus falling "correctly" (that is, no closer than distance d to the other gene) is therefore $\frac{m-2d}{m}$. We neglect here the very rare chance that the first gene may have fallen within a distance d of either end of the map; in this case the second gene is excluded from a space correspondingly less than $2d$ in length. The third gene, now, has a space of $m-4d$ in which it may fall "correctly," the chance of this occurrence being $\frac{m-4d}{m}$; for the fourth gene the chance, similarly, is $\frac{m-6d}{m}$, and so forth. Here we also neglect the slight source of inaccuracy due to the rare cases where two previously considered genes fall at a distance less than $2d$ apart from each other, leaving for the succeeding genes a slightly greater length of chromosome in which they may "correctly" fall than if the previous genes had been less crowded. This latter effect works in a direction opposite to that of the inaccuracy caused by possible terminal positions, but neither effect would cause a noteworthy error in data like those we have at present. The chance that all the n genes shall fall at least as far apart as postulated is now:

$$\frac{(m-2d)(m-4d)(m-6d)\cdots(m-2(n-1)d)}{m^{(n-1)}}$$

This can be reduced approximately to:

$$\left(1-\frac{d(n-1)}{m}\right)^n$$

The above formula, then, represents approximately the chance that none of the *n* mapped genes in a map *m* units long will fall as close together as the small distance *d*. Applying this formula to our best case—that of the approximately 25 well mapped genes in the 69 units of the X chromosome of *Drosophila melanogaster* that lie to the right of the obviously crowded region in the first unit at the left end—we find that, if *d*, the small distance considered, is taken as one tenth of a unit, our formula yields a chance of about one in three that no genes would be found so close together, *no matter how close together the undetected genes actually lay in the chromosome.* Our methods have not yet been delicate enough to distinguish gradations so fine as to lie between one and two tenths of a unit; the above result therefore means that, in our material, we had a very good chance (1 in 3), among only the 25 genes studied, of not finding any of them closer together than two tenths of a unit, even though the genes were as close together as 0.1 unit, or even if they were a million times as densely packed as this. The chance is even more against our finding closer linkage than 0.2 per cent in the autosomes, because of the difficulties in the way of the measurement of low linkage frequencies there. Thus our estimate of 1425, or 1800, genes, obtained by this method, even though it agrees fairly well with our estimate based on mutational recurrences, is only what we might readily have obtained by the random sampling of genes far more crowded, and it, like our other figure, may be a gross underestimate.

The above method of estimating gene number is also subject to other weaknesses, which make it unsafe even for the securing of a minimum estimate. One such defect is that the frequency of crossing over between two very closely neighboring loci may, for all we know, be somewhat different in the heterozygous condition—in which the determination of the short distance must be made—than in the homozygous condition, in which most of the chromosome existed when the determinations of its total number of units were made. If the crossing over frequency was lessened, locally, by heterozygosis, this would make the short distances appear spuriously short, in comparison with the total map length, and so the genes might be reckoned to be more crowded, more numerous, and smaller, than they really were. Again, there may be regional peculiarities of crossover frequency and gene spacing other than those responsible for the more obvious clusterings of the genes in the maps. The method based on crossover frequency is as yet, therefore, far less reliable, even for giving us an estimate of the smallest possible number of genes, than the method based on mutational recurrences, and for this reason the author has not, in previous publications, based any conclusions upon the results thereby obtainable.[3]

c. THE EVIDENCE FROM CHANGES IN BAR EYE

There is still another "straw in the wind," the significance of which for the problem here at issue has not hitherto been pointed out. It is, in a sense, a

[3] The first publication in which this method was put forward for obtaining an estimate of gene number was that of Morgan ('22).

special case of the above linkage method, but it has peculiar advantages of its own—as well as disadvantages. The matter in question is that of the so-called "mutations"—or crossings over—occurring at the locus of bar eye. Sturtevant has demonstrated that the supposed mutations of bar eye are nearly all of them really cases of askew crossing over. Now, if we assume that, in general, crossing over between the bar gene and its neighbors occurs with about the same frequency as crossing over ordinarily occurs between two neighboring genes, but that, here, the allelomorphs lie either opposed or askew, at random we find, on examining the data from Zeleny and from Sturtevant, that the bar gene is about one fifth of a unit from its neighbor.

These data, then, would again indicate a total of 1400 to 1800 genes in the haploid chromatin.

In this case we can be practically certain that we are dealing only with crossing over between directly adjacent loci (that is, loci without any other gene between them)—otherwise varying effects would have been produced by the askew crossing over, depending upon exactly which genes it happened to cut between. There are drawbacks in the present evidence, however, and that is that the crossing over relations at the bar locus are much disturbed when the bar genes are present, as shown by the asymmetry of crossing over, and so this may in itself entail an abnormal crossover frequency. It is also possible that the region containing bar lies close to the cytologically discovered "attenuated" section of the *X* chromosome. This might cause the frequency of crossing over to be unusually low in this region, as in the central portions of the autosome maps, and would correspond with the observation that the known loci in this region seem to show a tendency to clustering. To allow for such an effect, our estimate of gene number would have to be lowered.

d. CONCLUSIONS AS TO GENE SIZE

Taking, provisionally, the figure 1800, in the light of our three very different methods, as representing at any rate the minimum number of genes present, and dividing this into the haploid volume of chromatin, from 0.2 to 0.3 cubic micra, as determined by measurements of chromosomes, we obtain a maximum average gene diameter of approximately one twentieth of a micron. This is just below the limits of image formation by visible light, and in the range of size accepted for "colloidal" particles, but it might still contain hundreds of typical protein molecules. If, however, the actual number of genes is really much larger, if the gene is highly compound (see next section), or if the genes occupy but a small fraction of the volume of the chromatin—as is probably the case— then the real size of the elementary genetic unit becomes much smaller.[4] It should be pointed out

[4] It must be remembered that some sperm heads seem mainly composed of a combination of nucleic acid with protein of a relatively simple, *small* molecule type, with little (but probably enough) room left over for large protein molecules to be present in as great abundance as the genes seem to exist. There is, however, no strong reason to suppose either that the genes are composed of, or contain or form a part of, either simple protein molecules of nucleic acid, just because the major part of the chromosome substance is of this nature; on the other hand, it would be still more gratuitous to deny this possibility. At present any attempt to tell the chemical composition of the genes is only guess-

again, in this connection, that the above calculations deal only with the size of that portion of material, separable by crossing over, which is responsible for the transmission of an ordinary Mendelian difference. That difference might depend upon just a chemical radical or a single atom, or even upon a single electron position; at the other extreme it would also be possible that the ordinary Mendelian difference in higher organisms was a "regional" chromosome change, of variable extent, which generally covered much more than the minimal amount of substance capable of self-propagation in the nucleus.

Our formula, then, does not give us any direct information concerning the size of the smallest portion of gene material which would be capable of reproduction as such, without the coöperation of other, contiguous gene material. The small size of some "microchromosomes," and of some chloroplastid primordia, shows us, however, that the minimal amount of gene material, in this sense too, at most borders on the ultramicroscopic. We shall see later, moreover, that the primary mechanism of gene-propagation in all forms of life is probably identical with that in the simplest, smallest known organisms, and since in a virus we know this can be crowded into a space about a fiftieth of a micron in diameter the minimal amount of self-propagable substance is seen to be of ultramicroscopic dimensions. The similarity of dimensions of the quantity just mentioned to that above calculated for "maximal gene size" is interesting, although it may be accidental. Reasons will be given subsequently for the view that the smallest self-propagable unit is probably much smaller than this.

3. The Interrelations of Genes in the Chromosome

Although we may speak of genes as units, either in the sense of Mendelian differentiators or in the sense of independently propagable elements, it must be recognized that they may be only *potential* units, and that in the chromosome they may conceivably exist not as separate particles, or even as chemically disunited substances, but in the form of a linearly continuous structure. True, there is a tendency for a given differential to hold together as a unit when it crosses over aganist its allelomorph in the heterozygote, but we must also remember that in cases of mutation of an entire region, so-called "deficiency,"

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work. It is tempting, for instance, to suppose that genes are enzyme molecules, in special form, and that enzymes are proteins in special form, but while this appears more probable than any other individual guess, its chief merit after all is that it lies in the path of least mental resistance. Just because the molecular basis of the genes *does* multiply is the very reason why it is unnecessary to suppose that, in their reactions upon the cell protoplasm, these molecules, like those of enzymes, do not become used up. They may merely be reproduced as fast as they are used up. And the substances formed in these reactions may eventually *lead to* the production, or the modification, of the real enzymes. This speculation is not here urged as a probability, but merely as a warning against too ready acceptance of definite conclusions concerning gene chemistry. As we shall see later, the act of reproduction itself, which the gene carries out, must be designated as "catalytic," and in this respect it is indeed like the action of an enzyme, but its actual mechanism may or may not be like that of enzymes in general. In fact, "enzymes in general" are not yet known to constitute a chemically coherent group of substances.

this region, comprising numerous ordinarily separable loci, now holds together as a unit too, when crossing over occurs in the heterozygote. In the case of the unequal crossing over of bar eye, however, we can determine that the bar gene holds together as a unit even in the homozygous condition, for the askew crossing over here occurs just to the right or left of it, but never within it. Furthermore, in cases of chromosome breakage, fusion, or inversion of a region, such as is sometimes found in *Drosophila*, if the genetic material in the chromosome did not have some kind of segmental structure, then the rearrangement of its parts, at the point of breakage or reunion, should always be equivalent, in its effects, to what we now call a "gene mutation;" a number of cases are known in which no such effect has been observed, although in others "mutation" has apparently accompanied the rearrangement. More observations will be necessary on this point. Further important observations of Sturtevant on bar eye do suggest that the spatial relations of the genes with their neighbors are of genetic consequence, as well as their internal arrangements. In finding that two bar genes in contiguity in the same chromosome exert more effect than two lying in homologus chromosomes, he discovered that the local pattern of the genes is a fact which may itself become, in effect, genic in its significance. Nevertheless, this does not make improbable a segmental structure of the gene material.

If we accept the inferences, later to be drawn, that the self-propagable material everywhere is fundamentally alike, and that the smallest independently reproducing portion of it is exceedingly minute, then the chromosome must have some kind of linearly repetitive structure. It also would follow from the "gene-element" interpretation of eversporting genes, presently to be discussed, that the gene material at times at least may be non-linearly repetitive in its formation. Finally, the regularity of the mathematical relations observed in linear linkage, with its corollary, interference, proves that the disunion of parts of the chromatin structure from each other (in crossing over) is determined by geometrical and physical factors and not by chemical bonds or affinities of a sort that would differ from point to point according to the atomic configurations within the gene material. This fact too, then, connotes a chain-like, segmental arrangement of those atomic connections that must exist within the "chromatin" thread, allowing of breaks, between these segments, which are governed by "mechanical" circumstances.

Admitting as yet, however, the provisional nature of our conclusions regarding genic discontinuity, it is still quite legitimate to speak of the number or size of "genes," as suggested in the beginning, in the sense of the number or size of linearly connected[5] regions so taken that in them non-overlapping genetic differences, separable by crossing over, can occur, and these differentiable parts fit our conception of genes in being concerned in the propagation of their

[5] See Sturtevant's work on bar and infra-bar for proof that even two directly adjacent "genes" are linearly connected.

own specific structure largely independently of what other kinds of genetic material they may happen to be associated with.

4. Is the Gene Compound?

A converse of the above question of gene interrelations is that of its interior composition. If what we call the gene, in the above sense, can be shown to be made up of several or many identical parts (such as like molecules), then our most fundamental genetic unit becomes much smaller and our interpretation of basic genetic phenomena will be much affected in various ways. The work on eversporting, variegated plants, particularly the brilliant studies of Emerson, Anderson, Eyster, and Demerec, have, taken collectively, shown that here at least the gene is in all probability compound, since segregation of parts obviously occurs within it at some mitoses, but it is most important to know whether these cases afford a real glance into the interior of typical genes or are—if I may so put it—"teratological."

I mentioned in 1921 that the question might be solved if we could observe the distribution of mutant genes from the time of their origin, by noting the appearance or non-appearance of the mutant character in cells descended in a known way from the cell in which the mutation had occurred, as might conceivably be done in some somatic tissue. For, if the mutation originally happened in one of a number of interchangeable gene parts, or particles, then, as the particles of the new and the old kind, now existing side by side in the same gene, multiplied and became separated into two groups by chromosome division, followed by cell division, some cells would finally be formed having genes containing particles all of the mutant type, others particles all of the old type, just as, more grossly, green may become segregated from white plastids in leaf development, and—here is the important point—*these differing cells, forming areas of different genetic type, should dovetail amongst each other in the form of an irregular patchwork.* On the assumption of a gene compounded of equivalent particles, there appear only two alternatives to this eventuality, both of which, on analysis, turn out to be spurious.

The first apparent alternative would be that the collection of particles within the gene always divides by a kind of "equation division," so that the two gene-halves destined for the two daughter cells always contain a half of each gene-particle that the original gene had contained. In that case there would be no sorting out whatever, and the mutation, if it could produce any phaenotypic effect at all, could (subject to developmental influences) exert its effect from the beginning, on the whole block of cells derived from the cell in which the original mutation had taken place. In that case, however, with the passage of innumerable zygote-generations more and more of the gene particles within the gene in question would become differentiated from each other, through their separate mutations, until we had, in effect, as many different genes, of one particle each, which were individually capable of showing the typical phenomena of stable, Mendelizing differences. Hence this alternative destroys itself.

The second apparent substitute notion, for avoiding a patchwork effect, if the gene is compound, is that the original particles in the gene become sorted out, in subsequent cell divisions, in the same exact order of dichotomy in which they had originally been formed from one another within the gene. Thus the most closely related gene particles, and the group of particles descended from a mutated particle would come to lie in a group of related, adjacent cells, instead of becoming scattered irregularly in cell inheritance. Examining such a supposition further, we find that it would require an elaborate and orderly dichotomous grouping of the particles, bundle within bundle, in the order in which they had been formed in a hypothetical doubling and redoubling of the gene particles as the gene grew. This is shown in the figure. Now, as the chromosome split for mitosis, it would have to be supposed to divide first along the line of earliest separation (between the two largest bundles). In the gene growth following this division each particle would have to form exactly two again, and a new, as yet least-marked, line of division would form from between each of these two sister-particles, that became more prominent in each successive cell

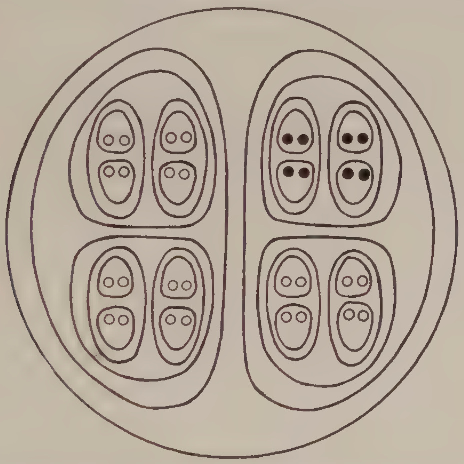

Fig. 1. Cross sectional diagram of chromosome,—showing the sort of dichotomous grouping of gene parts which would prevent the descendants of a single mutated gene particle (shown in solid black) from becoming scattered amongst non-continguous cells.

generation till it became the oldest division line, along which the split occurred. Since this set of conditions would have to apply to all the genes arranged linearly along the chromosome, we should probably have particles of corresponding descent arranged and held adjacent to one another in adjacent genes, so that all genes could undergo the same orderly splitting at the division of the chromosome. This would amount to our having the whole chromosome divided up into strands, bundle within bundle, in the same order as we supposed the gene particles to be grouped within the individual genes. In other words, we should really have, not so much multiple genes, as multiple chromosomes, longitudinally divisible and subdivisible finally into elementary strands. This notion seems to fall of its own weight, if carried beyond the stage of a few strands, but, in any

event, within each strand the gene would have but one particle (that is, no repetition of identical parts). Hence we see that the sorting out of descendants of a mutant gene-particle into just one block of cells(consisting of cells all more closely related to each other than to any others) would mean that the individual chromatin *strands*, at any rate, had a non-compound gene structure. A patchwork, on the other hand, would mean a compound gene, provided the cells remained together in order of their relationship.

I had at first thought that *Drosophila*, because of its "indeterminate cleavage," was unsuited for a study of the order in which mutant gene particles might be distributed in the embryonic cell divisions, but it occurred to me later that this feature need not make it impossible to obtain the desired evidence here, for, as the arrangements of parts in gynandromorphs showed, the cleavage nuclei usually preserve their original arrangements after division. Thus, although the first two nuclei sometimes are destined for the formation of anterior versus posterior halves, at other times for right versus left halves, or for somewhat diagonally cut fractions, their respective descendant nuclei seldom become irregularly scattered amongst one another, in the form of a patchwork. If, then, the gene consists of a number of particles, which do not maintain their order in the elaborate manner above detailed, the genetically differing parts of mosaic flies in which a mutation had occurred during early development should be scattered about irregularly in a much larger proportion of instances than is the case with male and female parts of gynandromorphs. But if the different parts do not have such a tendency to be scattered more irregularly than in gynandromorphs we could conclude the converse, that is, that the gene has not more than one particle, or at least not more than one particle in each strand of an orderly group of strands.

A survey of the rather meager number of somatic mosaics in *Drosophila* which had originated by gene mutation then indicated that in all these cases the mutated region seemed to form one complete block, contrasted with the complete block of non-mutated tissue. The clearly bilaterally divided yellow and gray male reported by me in 1920 is a good example of this. This, then, makes it seem likely that, in *Drosophila*, the genes in each chromosome strand are units, in that they do not contain more than one of any given kind of interchangeable, separately mutable, self-propagating molecules (or particles of any order).

In view, however, of the very remarkable results of Demerec, to be reported at the present meeting of the Congress, I must for a time reserve judgment, and admit the possibility that the mutational mosaics which more nearly resembled patchworks simply had not been detected, and that in the ones seen the superficial cells studied were already too far removed in descent from the original mutant ancestral cell to show the interdigitative effects of such intra-gene segregation. Or—as seems more plausible—it might be supposed that there is a partially orderly distribution of "gene elements," which only sometimes becomes irregular. Meanwhile, we must be prepared to watch closely for as many mutational mosaics as we can find, as well as to follow the

possible segregations of the gene particles from generation to generation of individuals—though previously it had not seemed to me likely that such segregation would still remain incomplete after an entire zygotic generation.

5. The Gene in Relation to Higher Life Processes

It may be reiterated at this point that there is no good evidence from genetics of any heritable differences between organisms except such as are resident either in the numerous genes of the nuclear "chromatin", or, much more rarely, in the plastid primordia, which may contain a homologous substance. And since our evidence is extensive, and the phenomenon of heritability a highly peculiar one, it is logical to conclude that in all probability all specific, generic, and phyletic differences, of every order, between the highest and the lowest organisms, the most diverse metaphyta and metazoa, are ultimately referable to changes in these genes—chiefly in the multitudinous and very diverse chromosomal genes, to a lesser extent in the plastid genes.

It is, to be sure, still contended, especially by a few paleontologists not familiar with genetics, that the "evolutionary" differences distinguishing species and other more widely separated groups are of another kind from the Mendelian gene-differences dealt with in ordinary crosses. But to these persons we may throw back the question: "Why, then, is it that we never *find*, in our crosses, organisms that differ in these other ways, the ways that are supposed to be of evolutionary significance"? Has evolution suddenly been stopped, somehow frozen, so that within species such differences are no longer accruing; or do the individual smallest steps out of which the larger differences are built always make the organisms so differing uncrossable? How convenient that arrangement would be for avoiding a genetic test of the issue! If so, why is it that the varieties of a species are so seldom found to be uncrossable (more often do we find intra-varietal or self-sterility, following Mendelian lines); do not crossable varieties represent "evolutionary" steps? As a matter of fact, many so-called "species" themselves are crossable, and, when crossed, they too give evidence that their differences are truly genic, of the kind dealt with above but very numerous. Are all such cases, then, to be outlawed, and put in a different category from those in which the species-building is of "true evolutionary significance?" Then indeed would nature have perpetrated a hoax on the geneticist. In that event certainly the correctness of an evolutionist's views would be measured by the extent of the faith which enabled him to hold to them, *despite all evidence to the contrary*. But fortunately such an attitude is now uncommon in science.

And so, even if the genes should not form the basis of the distinction between animate and inanimate matter in general (though I shall try to show later that they do), at least most biologists will now admit that they do form the bases of all the higher developments that distinguish the most advanced from the most primitive forms of life. And by the expression, "they form the bases," is meant that the change in the gene structure was the cause of the more superficially observable morphological or physiological difference, and that this same (thus changed) gene structure, now existing, is somehow necessary for the continued

maintenance of these observed properties, forms, and physico-chemical reactions. The genes are, of course, by no means the *only* necessary cause of the characteristics of the organism, any more than the present author is the sole cause of the present paper, but they are the *differential* causes of the specific, generic, etc., differences noted.

Just *how* these genes thus determine the reaction-potentialities of the organism and so its resultant form and functioning, is another series of problems, at present practically a closed book in physiology, and one which physiologists as yet seem to have had neither the means nor the desire to open!

6. The Gene in Relation to More Fundamental Life Processes

If the preceding discussions have seemed to carry us far afield, they are after all closely connected with actual data, the inferences therefrom are capable of concrete expression; the possibilities involved can largely be mapped out, and perhaps subjected to final testing by methods not unlike those we now possess. We may now allow ourselves to enter a realm further removed from our present experimental methods, devoted to basic questions that must as yet be treated in rather an abstract fashion.

We may put the questions: Could there ever have been a time, in the evolution of living matter, prior to the existence of what we may permissibly term the genes, and, at or before the time when genes did arise, how complicated could living matter have been, and what could have been its properties? We shall examine these questions only in the light of the naturalistic interpretation, that the complications of living matter evolved gradually, and that its operations were referable only to physical and chemical processes that were not somehow guided in advance by the relative advantages of alternative outcomes.

To start with, I think that most biologists will agree that we cannot speak of matter as "living" unless it has the property of growth, at least during a part of its career. In physico-chemical terms, growth involves "autocatalysis," inasmuch as the substances that grow must so affect some of the surrounding materials that the latter are transformed into end-products some of which are identical in composition with the former substances. Chemists ordinarily understand by an autocatalytic process any reaction, or set of reactions, in which one of the products formed (called the "autocatalyst)" tends to augment the speed of the reaction, and hence to cause the formation, within a given time, of more end-products, including, therefore, more substance like itself, than would otherwise have been produced. Thus, within that time, the "autocatalytic substance" causes an increase in the amount of material of its own kind. (This increase must, moreover, be greater than the amount of its substance used up.) Naturally, it is a not uncommon occurrence for the product of a reaction to influence, at least to some slight extent, either positively or negatively, the speed of that reaction, since few substances act with complete indifference in the presence of others that are undergoing recombination. Hence some degree of autocatalysis in the general sense above defined is by no means rare in chemistry.

In the growth of "living" substances, it is to be observed that a much more

peculiar type of autocatalysis occurs than the general type above described, since here it is not merely a question of the autocatalyst happening to affect the *speed* of reactions that are going on appreciably anyway. For "living" matter is not ordinarily produced, at least not to any appreciable extent, unless it is priorly present (else we should have "spontaneous generation.") Here, then, the "autocatalytic" substances—those that grow—may be considered as actually causing those particular reactions to occur whereby they themselves are produced.

When it is remembered that the medium surrounding living matter is necessarily complex, containing constituents that would render it ultimately capable of running through the whole multitudinous gamut of recombinations of organic chemistry, and that, on the other hand, the syntheses of "growth" must in any case be highly specific, it is seen to be a very noteworthy coincidence indeed that any organic autocatalysts should exist which happened to cause, among this array of possibilities, just those reactions which again produced their own particular physico-chemical structure. If, however, the fine distinction be made that possibly these reactions are not "caused" by the autocatalyst in an absolute sense, inasmuch as the reactions may actually be going on otherwise, but at an *inappreciable rate*, it must be observed also that the latter statement would apply equally well to innumerable other reactions, which also would be proceeding, at an inappreciable rate, amongst the substances initially present in a complex medium, but that the autocatalysts here in question must then be considered as specifically *selecting* that one amongst all these series of reactions, to catalyze, which happens to result in the products identical with themselves. Moreover, the addition of other substances to the medium, instead of the specific autocatalysts in question themselves, does not, in any case so far discovered, "cause" (in the above sense) those particular reactions to occur whereby these autocatalysts are produced. This emphasizes the special nature of the latter reactions, and therefore the degree of the coincidence that our growth-autocatalysts should be selectively "causing" just these reactions. It is in line with these considerations that, although autocatalytic reactions in the more general sense first defined are not uncommon in general chemistry, "specific autocatalysts"—which, in the above sense, are specifically necessary to "cause" the production of more material of their own kind,—are highly unusual outside of living matter,[6] since, regarded as a series of chemical events, such autocatalyses represent far more of a coincidence than the former ones.

We must note further that, in all such cases in ordinary chemistry, if we caused a change in the composition of the autocatalytic substance (through some further reaction that occurred after it had been formed) we should practically always render it non-autocatalytic. For the change will either leave the substance still capable of affecting the original reaction in the same way as before —in which case the substance will not be causing the production of material of

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[6] In these connections, the analogy of growth-autocatalysis to ordinary crystallization, as drawn by Troland, is suggestive, but it does not go far enough; moreover, if it did hold as pictured, we should expect much more evidence of gene-like action in "inorganic" material.

exactly its own kind any more—or, if the effect of the substance on the original reaction becomes altered, it would be most remarkable if it chanced to be altered in precisely such a direction that the reaction now caused was of just such a nature as to produce this substance in the exact form in which it had become modified. Such reciprocity between the change in the specifically autocatalytic substance and the change in the effect which it had on the substrate would require a special kind of construction in the former, of a sort not yet known to the chemists[6], which allowed of variation in certain features of its pattern while at the same time a mechanism was retained which caused that variable pattern to be copied in its present form by the substances being newly formed. A specifically autocatalytic substance of this kind would fulfill our definition of a gene, since it would be able to "mutate" without losing its property of "propagation." If able to mutate thus, indefinitely, it would eventually go through an evolution akin to biological evolution.

Now we shall attempt to show that, in the evolution of living matter, there was probably not a form of protoplasm, ancestral to our present protoplasm, which already had the power of growth (or "specific autocatalysis") without containing genes (that is, without that exceptional form of specific autocatalyst which is able to mutate and still retain its specific autocatalytic function, as we know a chromosomal gene can). If this is true, it means that "life" did not occur before the gene. And we shall attempt to show, further, that this first material, ancestral to our own, that could grow, probably consisted of little else than the aforementioned gene, or genes.

In the first place, attention should again be called to the fact that the more complicated a given chemical product is, the more special will be the reactions necessary to produce it, and therefore the slimmer and slimmer (in geometrical progression) will be the chance that this product will happen itself to produce just these same reactions, that is, to be "specifically autocatalytic" (unless we either suppose some purposive organization of the external medium in adaptation to it, or suppose a gene-mechanism within it that built up the complications through a series of *selected* mutations). Thus it is almost inconceivable that a whole system of substances as complicated as that which any protoplasm or cytoplasm is conceived to be, should have come together by the chance action of physical and chemical substances, to form just such a structure that the working of this system reproduced that same system again, in all its features—*unless* the whole mechanism of biological evolution, involving reproduction, variations, the reproduction of variations, and natural selection, had long previously been at work building up this system in just the right way from much simpler beginnings, that is, unless substances that may, in effect, be called genes, had existed first.

The difficulty involved in the production of any such system, antecedent to the existence of *mutably* autocatalytic bodies—that is, genes—becomes still more insurmountable when we consider that the highly adapted system thus thrown together would, on one view (1), have to have the property not only of reproducing its protoplasmic portion—growing, if you prefer—but, in addition,

it would by chance have to possess in perfected form before their use properties like those of a mimeograph. For when (perhaps much later) the mutable genes came into existence in connection with this protoplasm, the latter would have to have the effect of reproducing these genes too, each after its own mold, somewhat as a mimeograph reproduces forms presented to it. An alternative (2)—if we should still hold to the hypothesis in question that the protoplasm arose without the coöperation of preëxisting genes—would be to ascribe to the composition of the genes themselves most of the mechanism necessary for their mutably specific autocatalytic properties, but such a double origin of the remarkable attribute autocatalysis, in both protoplasm and genes independently, is doubly improbable—or, should we say, improbable as the square, especially in consideration of these two autocatalyses being of fundamentally different types, immutable in the protoplasm, mutable in the genes. Thirdly (3), we cannot well derive the genes from the protoplasm, by some sort of mutation, in order so to explain their specific autocatalytic ability, because we have not supposed the protoplasm to be able to mutate without *losing* its autocatalytic ability. If it had been able to do so, it would itself fulfill the definition of "gene material" and so we should after all have had the genes to begin with. Moreover, in that case there might be evidence of the existence, even today, of material in the cytoplasm having the potentialities of genes—that is, propagable despite mutation. Any intermediate position between the three hypotheses noted above will simply share, in appropriate ratios, their respective difficulties.

We are, therefore, practically driven to the conclusion that the extraordinary property of protoplasmic growth, or reproduction—biological autocatalysis—did *not* exist before the property distinctive of the gene existed—namely, before that peculiar form of specific autocatalysis existed which is compatible with change in composition (mutation). The gene, then, arose coincidentally with growth and "life" itself, if our argument be correct.

We shall further conclude that at the time of its inception this mutable autocatalytic system was extremely simple, as compared with forms of protoplasm that have as yet been analyzed, consisting of little or nothing else, in fact, than what may be called the gene. For the more complicated we imagine any chance-supplied companion-structures (corresponding to the general "protoplasm") to have been, which were supposedly necessary in the first place for the operation of the genic autocatalysis, the more we run into difficulty in trying to account for the lucky coincidence that the combined activity of the genes and of these necessary by-standing substances should have caused the reproduction, *not only* of the genes, but also of these other materials themselves. Similar considerations to those followed here also lead us to the view that probably the gene itself was not highly complicated in its essential structure, inasmuch as it had to be specifically autocatalytic in respect to all its parts.

What the structure of the gene itself actually was, in physico-chemical terms, the modern geneticist would like to know—perhaps beyond all other questions of genetics—but as yet he remains in almost complete ignorance of this matter. What feature or features of its structure allowed it to *mutate* without losing this

specific autocatalytic ability can only be guessed at most inadequately now, but in these features lay all the promise of life, as distinguished from the inanimate. The mutations must have been, must still be, rearrangements in pattern of one sort which leave unchanged certain other arrangements, of an entirely different sort, which are responsible for the specific autocatalysis. The latter, *stable* arrangements somehow result in the copying of the former, mutable arrangements (as well as their own), by the raw material as it becomes organized, and the character of these mutable arrangements, in turn, is of great moment in determining those *not* directly autocatalytic reactions of the gene with its surroundings, whereby it now organizes its environment in its own interests.

The latter, mutable sorts of arrangements within the gene material have certainly undergone vast changes and complications in the course of evolution, and under their influence, of course, the protoplasm has evolved and changed, but the other arrangements, those features of gene structure which are responsible for its primary autocatalysis—these must still be the same as in the immemorial ages past, before green slime bordered the seas. The secret of this immutable (but mutation-permitting) autocatalytic arrangement of gene parts may perhaps be reached first by an upward thrust of pure physical chemistry, or perhaps by biologists reaching down with physico-chemical tools through the chromosome, the virus, or the bacteriophage. Studies of the nature of gene-attractions may help. This matter lies, as it were, in another dimension from mutation—and perhaps this statement may be true even literally speaking.

When we take this point of view, it is evident that we escape our logical difficulty concerning the origin of present-day protoplasm, with its intricately interlocking parts that all act to further the growth and exact reproduction of the whole. For the origination of this system, just like that of the complicated gross morphology of the higher plants and animals (which was a later accomplishment of the gene), came about gradually, step after tested step, as mutation followed mutation in the primordial autocatalyzing genes. In this process those mutant genes whose by-products (end-products other than their own material, not originally necessary for "life") were most useful in further reproduction differentially survived, multiplied, and mutated again. On this view, then, the view that seems best to stand the tests of ultimate analysis, the great bulk, at least, of the protoplasm was, after all, only a by-product, originally, of the action of the gene material; its "function" (its survival-value) lies only in its fostering the genes, and the primary secrets common to all life lie further back, in the gene material itself.

Even now, in a large cell, which (in diploid) contains thousands of genes, a change in a single invisible one of these ultra-microscopic particles can profoundly affect the physical and chemical properties of the entire mass, and the resultant physiological reaction-systems are distant reflections of the new gene-composition. The mutations of the genes have thus laid the building blocks of the present protoplasm even as they hold hope for its still further improvement.

7. The Attack Through Studies of Mutation

How little critical work has been done in the study of these all-important mutations, as compared with the work on what we may now term the superficial aspects of organisms! Through the work on gene mutation we may not only gain knowledge of the mode of occurrence of the changes themselves, leading possibly to some degree of control over protoplasm from its root, as it were, but we may perhaps also gain some insight into those genetic arrangements which are subject to this mutation. As will be explained in the papers of Eyster and Demerec, most instructive work is now being done on "eversporting" plants and animals, which throws light on the possibly compound nature of the gene as it exists in them. I should, however, prefer to call these cases of *eversorting*, rather than *eversporting*, genes, since we seem to be dealing with a phenomenon of intra-genic segregation of particles already differentiated by mutation. This still leaves open for study the problem of the original causation of mutation in whatever may be the elementary gene particles.

On the latter question, that of the causation of the primary mutations, there has—with one exception—been only negative evidence as yet, all agents tested in the attempt to produce mutation having visibly failed. Recent experiments of the present author, however—especially when taken in connection with some prior work done in collaboration with Altenburg, and some work done with Hanson, have indicated that a rise in temperature increases the likelihood of mutation in *Drosophila*. This is only a first step in a laborious quest, but the elaboration of special genetic methods and stocks in *Drosophila*, which allow the detection of lethal factors in relatively great abundance, now makes such work on mutational origins feasible for the first time. The beginning of the pathway to the microcosmic realm of gene-mutation study thus lies before us. It is a difficult path, but, with the aid of the necromancy of science, it must be penetrated.

We cannot leave forever inviolate in their recondite recesses those invisibly small yet fundamental particles, the genes, for from these genes, strung as they are in myriad succession upon their tiny chains, there radiate continually those forces, far-reaching, orderly, but elusive, that make and unmake our living worlds.

Résumé

1. The method is explained whereby a minimum estimate of the number of genes in *Drosophila* may be obtained from data showing the observed frequency of occurrence of mutations in loci identified as separate. The (haploid) number based on recent data is about 1150. Both rough and more refined approximation methods are described. It is proved mathematically that the number of genes so arrived at is truly minimal, probably far below the actual number, and it is shown that more nearly correct (though still minimal) numbers might be obtained by such methods if extensive data on autosomal lethals were at hand.

2. The attempt to estimate the number of genes by dividing the frequency of crossing over between genes as nearly adjacent as possible into the total

frequency of crossing over is criticized. It is shown mathematically that the results obtained could have been produced even if the genes were indefinitely more numerous than they are calculated to be by this method, but other elements of uncertainty, concerning the relative frequencies of crossing over in different parts of the chromatin, and under different genetic conditions, make possible the other alternative, that the estimate so arrived at is too large. The number of genes calculated by the method here discussed is between 1400 and 1800.

3. It is shown that the data on symmetrical and asymmetrical crossing over between the "genes" for bar eye, obtained by Sturtevant, furnish a special case of the second method, with the peculiar advantage that the crossing over is known to be between adjacent loci. This method gives a figure similar to that obtained by the second method.

4. When the "minimal number" of genes is divided into the bulk of the chromatin containing them (as measured by Bridges) it is found that the "maximal" diameter possible for the average gene is about a twentieth of a micron, just within the range of size of ultramicroscopic, colloidal bodies. The real size may be much smaller, however, and we have as yet no clue to possible gene chemistry.

5. Various considerations make it probable that the genes in the chromosome are not in the form of a continuum, but occur segmentally, in units the interconnections of which are like one another, and different from their intraconnections. Nevertheless the spatial arrangements of the genes with regard to one another may have some influence upon the effects which the genes produce in the cells.

6. The arrangement of parts in mosaics of *Drosophila* caused by mutation furnishes evidence indicating that the gene is not composed of identical particles which ordinarily may undergo rearrangement in the formation of the daughter-genes. It is therefore indicated that a gene in a given chromosome-strand does not contain more than one molecule of a given kind. However, in the face of the apparently contrary evidence of Anderson, Eyster, and Demerec on eversporting organisms, more data will be necessary to decide whether or not the "typical" gene is composed of a number of identical parts that may *at times* become re-arranged.

7. It is pointed out that growth involves a specific autocatalysis, without which life cannot exist. The gene, when in its protoplasmic setting, is known to possess this property of "specific autocatalysis." Still more remarkable, the gene can mutate without losing its specific autocatalytic power. In view of this latter peculiarity of the gene, it becomes a supererogation, and involves improbable assumptions, to suppose that any other portion of the protoplasm, independently of the gene, is specifically autocatalytic; "growth" of the rest of the protoplasm would follow as a by-product of gene activity. Likewise it involves apparently insuperable difficulties to suppose that, in the most primitive living matter, highly organized companion substances to the gene ("protoplasmic" in nature) were necessary to make the gene-autocatalysis

possible. Genes (simple in structure) would, according to this line of reasoning, have formed the foundation of the first living matter. By virtue of their property (found only in "living" things) of mutating without losing their growth power they have evolved even into more complicated forms, with such by-products—protoplasm, soma, etc.—as furthered their continuance. Thus they would form the basis of life.

8. Modern work on mutation holds promise of attacking successfully some of the important problems concerning the gene. Such studies are now feasible for the first time, and a beginning has already been made, in the finding that temperature affects the rate of mutation.

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THE BEARING OF VARIEGATIONS ON THE NATURE OF THE GENE[1]

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Introduction

Variegations are especially favorable material for the study of the nature of the gene, because they are extremely variable and give rise to genetic variations which behave in inheritance as multiple allelomorphs. The occurrence in variegations of an orthogenetic series of genetic variations, which range from one extreme condition to an opposite condition, suggests that the gene itself undergoes corresponding quantitative changes. The purpose of the present paper is to show that the gene, which commonly behaves as a unit in inheritance, is actually made up of more elementary genetic units which I have previously called *gene elements*. With this conception of the gene, the origin and inheritance of the frequent and quantitative variations which are common in variegations become clear and understandable.

The Inconstancy and Quantitative Nature of Variegations

De Vries ('05) was among the first to call attention to the extreme variability of variegations in plants. He studied a variety of *Antirrhinum* with variegated flower color in which he found a continuous range of variation from narrow- to broad-striped patterns. The plants with variegated flowers, the narrow-striped less frequently than the broad-striped patterns, produced occasional self-red flowers.

Correns ('10) reported a study of a chlorophyll variegation and a flower-color variegation in *Mirabilis Jalapa*. The variegated-leaved plants were found to give rise to self-green bud sports, and the plants with variegated flowers produced occasional self-red flowers. In each case the somatic change proved to be heterozygous for the self-colored condition and the variegation from which it arose.

Emerson ('13, '17) showed that self-red areas of all sizes occur in maize with variegated pericarp. The proportion of self-reds that any variegation produces was found to depend roughly on the amount of red in the variegated kernels. The self-colored ears so produced were found to behave as if they were F_1 hybrids between self-colored and variegated or between self-colored and colorless races, depending upon whether the variegated parent ear was homozygous or heterozygous for pericarp color and upon whether it had been self- or cross-pollinated.

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Self-colored ears were also occasionally found with the normal variegated ears in F_1 of a cross produced by pollinating a colorless race with pollen from a variegated race; and these F_1 ears always behaved in later generations as if they were hybrids between self-colored and colorless races.

Emerson pointed out also that variegations not only give rise to self-colors but also to variegations of other patterns. The existence of the series of at least nine or ten multiple allelomorphs to which variegation belongs was considered evidence that a factor for pericarp color has mutated repeatedly. Some of these factors appear to be stable, while others have mutated rarely and still others many times. Emerson considers the principal difference between certain factors to lie in their relative frequencies of mutation.

Hayes ('17) found *mosaic* pericarp in maize, a very coarsely variegated type, to be even more variable than the *calico* type of variegation studied by Emerson. Self-fertilization and selection within a red mosaic pericarp pattern color of maize isolated self-red, variegations of various patterns, dilute reds (patterns), and colorless types, each of which bred relatively true. These types were shown to form a series of multiple allelomorphs.

The writer (Eyster '24) studied the dilute red pericarp which occasionally arises from the calico type of variegation studied by Emerson. The dilute reds, which were called oranges in my previous publications, were found to vary quantitatively in color from red to whitish, and to give rise to variegations ranging from extremely heavy patterns, with many red areas on each kernel, to extremely light patterns, with sometimes only a single streak of color apparent on an entire ear. Dilute reds of different intensities and many quite distinct variegation patterns originated in pedigreed cultures from a single dilute red kernel and were found to behave in inheritance as a series of multiple allelomorphs. All color and pattern changes were found to be reversible and to occur with different frequencies.

A similar study (Eyster '25) was made upon the mosaic pericarp previously studied by Hayes. From a single parent kernel were derived, in pedigreed cultures, dilute reds varying from red to whitish and mosaic patterns ranging from extremely coarse and heavy types, with much pigment in the pericarp, to light patterns, with sometimes only one or a very few splashes of color in the pericarp of an entire ear.

De Vries ('10) found the flower color of a variety of *Hesperis matronalis* L. to vary from violet, through lilac of various intensities and variegations of different patterns, to colorless.

Baur ('24) reported a similar series of variations in the flower color of *Antirrhinum*. The petals were found to vary in color from red, through dilute reds of different intensities including pale red, flesh color, pale flesh color; and to be self-colored or variegated in different patterns, or colorless (ivory). These various colors and patterns form a series of multiple allelomorphs.

At the present time are growing in the flower garden of Prof. F. J. Kuney, of the University of Maine, extensive plantings of candytuft, *Iberis umbellata*, which originated from a common source. The flower colors vary from pansy

violet, through various shades of purple, lilac, and pink, to white. There also occur a number of distinct variegations. Tests have not yet been made to determine the genetic relationships of these flower-color variations in candytuft.

It is a significant fact that what appear to be similar and parallel genetic changes occur in both the calico and mosaic types of variegations in maize, in petal color of *Hesperis matronalis*, in petal color of *Antirrhinum*, in petal color of *Iberis umbellata*, and doubtless in other similar variegations. The common origin and allelomorphic relationships of a similar series of quantitative variations of different variegations indicate that a gene has undergone corresponding quantitative changes and that the fundamental nature of the gene changes is common to all variegation series.

Frequencies of Color Changes in Heterozygous and Homozygous Dilute-Red Pericarp

In a previous study (Eyster '24) 57,167 dilute-red (orange) kernels heterozygous for colorless pericarp were examined for color changes to darker red and to lighter red or colorless respectively. Only changes which covered one-eighth of a kernel and larger areas were recorded. There were observed 597 such color changes to darker red, and 407 changes to lighter red and colorless. These results suggested that changes to darker and lighter colors occur with approximately equal frequencies in pericarp having a gene for pigment associated with a gene for colorless pericarp.

To make a further study of the frequencies of color changes in dilute-red pericarp, a strain homozygous for dilute-red pericarp and cob, of the genetic constitution $\frac{DD}{DD}$, was crossed with a plant having colorless pericarp and red cob, of the constitution $\frac{WR}{WR}$. When the F_1 plants, of the constitution $\frac{DD}{WR}$, having the dilute-red pericarp of the one parent and the red cob of the other, were self pollinated, the F_2 progenies consisted of three kinds of ears in the ratio of 1:2:1 as follows: (1) dilute-red ears with dilute cobs, of the constitution $\frac{DD}{DD}$, (2) dilute-red ears with red cobs which are heterozygous for dilute-red pericarp and cob and colorless pericarp and red cob, of the constitution $\frac{DD}{WR}$, and (3) ears with colorless pericarp and red cob, of the constitution $\frac{WR}{WR}$.

The cob color, which is dilute-red in ears homozygous for dilute-red pericarp and red in ears heterozygous for dilute-red pericarp, made it possible to distinguish between the homozygous and heterozygous dilute-red ears in the same F_2 progeny. Among both homozygous and heterozygous ears, but more in the latter than in the former, there was noticeable variation in the color intensity

of the dilute-red pericarp, as has already been pointed out elsewhere (Eyster, '24, Table 1). In all the F_2 progenies grown the dilute-red pericarp is noticeably lighter in the heterozygous ears where the dilute-red gene is associated with a gene for colorless pericarp than in pericarp that is homozygous for dilute-red pericarp.

Pedigree 6765 was F_2 progeny from a cross between dilute-red pericarp and colorless pericarp, and consisted of 32 homozygous dilute-red ears, 68 heterozygous dilute-red ears, and 33 colorless ears. The homozygous and heterozygous dilute-red ears were separated on the basis of cob color and examined for color changes extending over more than a single kernel. None such was found. The kernels were removed from each group of ears separately. A sample of several thousand kernels of each group was closely examined individually for color changes to darker red, on the one hand, and to lighter red and variegations, on the other, with the results given in table 1. Each color change was tabulated, according to its size, as a *line*, when it formed a continuous line from the tip to the base of a kernel; as a *band*, when it covered approximately one-sixteenth of the surface of a kernel; as $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ of the surface of a kernel; and as 1 when it extended over the surface of an entire kernel.

TABLE 1. COLOR CHANGES IN DILUTE-RED MAIZE PERICARP (A) HETEROZYGOUS FOR COLORLESS PERICARP RED COB, AND (B) HOMOZYGOUS

	Color changes expressed in fractional parts of the surface of a kernel												Total number kernels examined
	Darker red					Lighter red and variegated							
	Line	Band	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	Line	Band	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$		
(A) Heterozygous pericarp............	1554	84	13	7	4	1556	92	13	9	4	3		3591
(B) Homozygous pericarp............	371	20	8	4	1	1076	78	20	4	3	0		4047

The 3591 heterozygous dilute-red kernels examined had 1544 lines of darker color and 1566 lines of lighter color, a close approximation to equality. The same relationship between changes to darker and lighter colors was found among those which covered larger areas, of which there were 84 bands of darker and 92 bands of lighter color, 13 darker and 13 lighter areas covering one eighth of a kernel, 7 darker and 9 lighter areas covering one-fourth of a kernel, 4 darker and 4 lighter areas extending over one-half of a kernel, and 3 darker and no lighter areas extending over three-fourths of a kernel. In all there were 1662 color changes to darker red and 1697 color changes to lighter red and variegation.

Even a superficial examination of the homozygous dilute-red kernels gives evidence of a higher frequency of change to lighter red and colorless than to darker red. On 4047 homozygous dilute red kernels examined were found 371 lines of darker and 1076 lines of lighter pericarp, 20 bands of darker and 78 bands of lighter, 8 darker and 20 lighter segments covering one-eighth of a kernel, 4 darker and 4 lighter color changes covering one-fourth of a kernel, and 1 segment of darker and 3 segments of lighter that extended over as much as one-half of a kernel. These data indicate that in the kernels studied dilute-red pericarp,

when heterozygous for colorless, changed to lighter colors approximately 3 times as often as to darker colors. In all there were 404 color changes to lighter and 1181 color changes to darker colors. This is a ratio of 1:2.92.

The above comparison shows that dilute-red pericarp heterozygous for colorless changes to darker and lighter colors with approximately equal frequencies, while homozygous dilute-red pericarp appears to change to lighter colors nearly 3 times as often as to darker colors. In order to be absolutely certain about the relative frequencies of changes to darker and lighter colors in dilute-red pericarp it will be necessary to study lighter intensities of red than are now available. The frequency of change to darker colors is about 4.6 times as great in heterozygous as in homozygous dilute-red pericarp. These results are quite in line with the very interesting and significant observation made by Emerson ('22), who found that changes to red in the calico type of variegation occur more frequently in heterozygous than in homozygous variegated ears of the same F_2 progeny. The difference was found to be especially pronounced in very light variegated strains, where mutations (areas of self-color) have appeared about 2.5 times as often in heterozygous as in homozygous variegated ears.

On the basis of previous studies and of the new data presented in the present paper, a simple hypothesis has been formulated as to the nature of the gene, and the nature and mechanism of the quantitative genetic variations that occur in a variegation series, and doubtless in other series of quantitative variations.

Nature of the Variegation Gene

To account for the frequent occurrence of self-green leaves and branches on variegated-leaved plants and of self-red flowers on plants with otherwise variegated flowers, Correns ('10) assumed that the gene for variegation has associated with it a latent gene for self-color. At more or less frequent intervals the self-color gene became separated from the variegation gene, and when this happened a self-colored area would be produced, its size depending upon the stage in development at which the segregation occurred. A part of the self-color gene material was thought to be retained by the variegation gene which then regenerated another self-color gene which would later segregate from the variegation gene. Thus he accounted for the frequent and irregular occurrences of self-colored areas on variegated plants.

The change from variegation to self-color in maize pericarp has been referred to by Emerson ('13, '17, '22) as sporophytic mutation, which differs from other point mutations primarily in the higher frequency of mutation. At one time Emerson suggested that the gene for variegation, *V*, may be in the nature of a temporary, recessive inhibitor that sooner or later permanently loses its power to inhibit color development, becoming thereby a gene for self-color, *S*. Or that the gene for self-color, *S*, may be temporarily inactive, but sooner or later becomes permanently active.

No student of variegations can long resist the idea that the quantitative

changes in the soma are the expressions of corresponding quantitative changes in the fundamental gene responsible for the variegation series. Küster ('18) pointed out that the shape of spots in variegations, their sharp limitations, and often also the arrangement of their cells suggest that they took their origin from one initial cell, which in turn arose by an unequal division. This unequal division was regarded as physiological rather than morphological and as the cause of different methods of reaction to external influences. More recently Küster ('19) suggested that the phenomena of variegations can be most simply explained by assuming reversible somatic mutations, a view earlier expressed by Emerson.

Nagai ('24) reported some observations on somatic segregation in soybeans and suggested that there may be a relation between the quantitative difference of phenotypic expressions and the respective differences in the quantity of the material which composes the genes. He considered it safe to define a mutation as a permanent rearrangement in the distribution of the material composing the gene.

In an earlier publication (Eyster '24) the writer suggested that the quantitative variations in a variegation series in maize pericarp, including a number of reds, dilute-reds of different intensities, variegations of different patterns, and colorless could be most easily explained by assuming that the gene for dilute-red (orange) pericarp is a compound structure, made up of pigment-producing and non-pigment-producing *gene elements*. According to this view the intensity of the dilute-red color would depend upon the relative numbers of the contrasting gene elements incorporated in the structure of a particular gene. The most striking as well as the most significant feature of dilute-red pericarp, it was pointed out, is its frequent replacement by equal and adjacent segments of darker red and lighter red or colorless tissue (Eyster '24, Plate I, C). The occurrence of parallel and adjacent segments of red and colorless tissue on otherwise dilute-red kernels was considered evidence that the gene which had been producing dilute-red pericarp had divided into daughter genes in such a way that one of them included an excess of the pigment-producing gene elements while the other had a corresponding excess of the non-pigment-producing elements. When this somatic segregation extended over the entire kernel or ear, the orange pericarp became changed thereby into a variegation.

All of these conceptions of gene changes are alike in that they are based upon general observations instead of actual data, and in no case is a method given, or even suggested, of putting the hypothesis to an actual test.

Further data are now available, and being in general accord with my previously expressed conception of the gene, they justify its formulation into a simple hypothesis which can be subjected to crucial tests. The general hypothesis follows:

The gene is made up of a definite number of gene elements which may or may not be of the same physical, chemical, or physico-chemical nature. Let *a* represent a gene element for pigmented pericarp, and *b* a gene element for colorless pericarp. The unstable genes of any variegation series have both *a*

and b elements incorporated in their structure. If the number of a elements in a particular gene is x and the number of b elements is y, the total number of elements would be $x+y$, which is equal to k. On the one end of the series, x may become equal to k when y equals zero so that the gene is composed entirely of a elements and has the general stability of genes made up of the same kind of gene elements. As the number of a elements decreases, the number of b elements increases until at the other end of the series y may become equal to k when x becomes equal to zero. Accordingly the formulae of the genes belonging to a variegation series may be expressed as follows:

$>xa+<yb$ = self-red pericarps, with the intensity of red increasing as x approaches k in value.

$xa+yb$ = dark shade of dilute-red.

$(x-1)a+(y+1)b$ = lighter shade of dilute-red.

$(x-2)a+(y+2)b$ = still lighter shade of dilute-red.

. . . .

$(x-n)a+(y+n)b$ = lightest shade of dilute-red.

$(x-n-1)a+(y+n+1)b$ = colorless pericarp, giving rise to a heavy variegation due to the frequent occurrence of genes with more than $x-n-1$ of the a elements, as the result of the chance assortment of the gene elements at each somatic mitosis.

$(x-n-2)a+(y+n+2)b$ = colorless pericarp, giving rise to a lighter variegation because of the less frequent occurrence of genes with more than $x-n-1$ of the a elements.

$(x-n-3)a+(y+n+3)b$ = colorless pericarp, giving rise to a still lighter variegation because genes with more than $x-n-1$ of the a elements are produced still less frequently.

. . . .

$(x-n-m)a+(y+n+m)b$ = colorless pericarp, giving rise to the lightest variegation because only rarely does a cell receive a gene with more than $x-n-1$ of the a elements.

$(x-n-m)a+(y+n+m)b$ = colorless pericarp, which becomes incapable of giving rise to variegations as the number of b elements approaches the value of k.

It follows then that red pericarps of different intensities ranging from deep red to whitish, variegations of different patterns, and colorless pericarps form a single series of quantitative variations ranging from the deep reds which are the expressions of genes made up of a relatively large number, or entirely, of the pigment-producing a elements, and correspondingly few or none of the non-pigment-producing b elements, to the colorless pericarps, which are the expressions of genes with relatively few or none of the a elements and many or entirely of b elements.

It has been shown that dilute-red pericarp heterozygous for colorless pericarp changes to darker and lighter colors with approximately equal frequencies. This is in accordance with expectation, for whenever a gene for dilute-red of a certain intensity divides unequally, daughter genes will be produced which have

a relatively larger number of *a* elements and a correspondingly smaller number of *b* elements, on the one hand, and a smaller number of *a* elements and a correspondingly larger number of *b* elements, on the other; thus tending to give rise to color changes to darker red and to lighter red or colorless, respectively, with equal frequencies. If each gene element of a gene for dilute-red pericarp of the constitution $(x-4)a+(y+4)b$, were to reproduce itself and then all elements were to be divided at random into two daughter genes of the constitutions $xa+yb$ and $(x-7)a+(y+7)b$ respectively, there would be corresponding changes to darker and lighter colors in the soma.

In homozygous dilute-red pericarps the changes to lighter colors were found to be approximately three times as great as changes to darker colors. These results are in accordance with expectation if the order of dominance in genes with fewer than x of the *a* elements tends to vary inversely with the number of elements and directly as the number of *b* elements in a gene. When a gene contains *b* elements exclusively it behaves as a recessive to all other genes. With this order of dominance a color change to lighter would occur whenever a cell receives the one, the other, or both of the daughter genes with a smaller number of the *a* elements, while a color change to darker could occur only when both of the genes divide unequally and the two daughter genes, each having a larger number of *a* elements, become incorporated in the same cell at the following mitosis.

Unfortunately, the dominance relationships of the different reds, dilute-reds, and variegations in maize pericarp are not well known, because intercrosses have been avoided in the past on account of the inconstancy of each type. When dilute-reds or variegations were crossed, usually some stable allelomorph, as colorless pericarp red cob or colorless pericarp white cob, was used as one parent. If among the genes for dilute-red and variegated pericarp those with few *a* elements and many *b* elements are more or less dominant over genes with more *a* elements and fewer *b* elements, light variegations should tend to be dominant over heavy variegations and variegations should tend to be dominant over the dilute-reds. Such data as are available on these relationships will be presented later in this paper.

Reference has already been made to the observation by Emerson that a larger number of colored areas occur in the pericarp of heterozygous than of homozygous variegated ears of the same F_2 progeny. At this point it must be recalled that a variegation is produced by a gene which has the contrasting gene elements in such proportion as to produce colorless pericarp, but by the chance assortment of its constituent *a* and *b* elements cell progenies covering areas of different sizes result which have sufficient of the *a* elements and correspondingly fewer of the *b* elements to develop pigment. In heterozygous variegated pericarp, daughter genes with more *a* elements and genes with fewer *a* elements respectively will be produced in approximately equal numbers. Any gene which receives enough of the *a* elements to develop pigment is free to express itself because it is associated with a recessive gene for colorless pericarp. The rate of change to pigment-producing genes may be expressed as one-

half. There is a similar change toward colorless, but as the pericarp is already colorless there can be no expression of changes in this direction.

In homozygous variegated pericarp both of the pericarp genes are dividing to form daughter genes with more *a* elements and fewer *a* elements respectively, exactly like the gene in heterozygous pericarp. A colored area will be produced only when a cell receives two daughter genes each of which has enough of the *a* elements to produce pigment as a result of coincident changes and somatic segregation in both genes. Such coincident segregations should occur in approximately one-fourth of the somatic mitoses, so that the frequency of the occurrence of the colored areas may be expressed as one-fourth. When the one, the other, or both daughter genes with a smaller number of the *a* elements go to a daughter cell there is a similar change towards colorless, but as the pericarp is colorless such changes can not be expressed.

Accordingly, colored areas should occur in heterozygous variegated pericarp with a frequency of one-half, and in homozygous variegated pericarp with a frequency of one-fourth. That is to say, colored areas should occur twice as frequently in heterozygous as in homozygous pericarp. The results reported by Emerson ('22), who found that colored areas have appeared about 2.5 times as frequently in heterozygous as in homozygous material, are in fairly close agreement with this expectation.

Crucial Tests of the Hypothesis

The conception of the nature of the gene which has been outlined above has the advantage that it may be subjected to crucial tests. The self-propagation of the individual gene elements and the random assortment of the elements as a gene divides for each somatic mitosis furnish the mechanism for the segregation of contrasting characters *in the soma in the course of development* according to expected ratios, which are just as definite as and quite similar to the Mendelian ratios in the F_2 progenies of crosses between individuals with contrasting characters. The segregation of contrasting gene elements in the same gene gives rise to somatic segregation of contrasting characters in the soma of an individual organism, while the segregation of entire genes in sporogenesis or gametogenesis gives rise to the segregation of individuals with contrasting characters or Mendelian segregation.

It is obvious that variations in a variegated series are determined by the segregation of entire genes in sporogenesis, by the recombination of entire genes at fertilization, and by the random assortment of the individual elements of a gene at each somatic mitosis.

In order to make a more careful study of the somatic segregation of darker and lighter colors in heterozygous and homozygous dilute-red pericarp, an F_2 progeny from the cross dilute-red pericarp and cob × colorless pericarp red cob, grown under pedigree 6766, was used.

The heterozygous ears had red cobs and the homozygous ears had dilute-red cobs, and this difference made it possible to separate them. The heterozygous

ears were noticeably lighter than the homozygous ears and also covered a wider range of color intensities, as indicated in table 2.

TABLE 2. COLOR INTENSITY OF (A) HETEROZYGOUS AND (B) HOMOZYGOUS DILUTE-RED EARS OF THE SAME PROGENY

	Whitish..........					.Dark dilute-red	
	a	b	c	d	e	f	g
A..............	1	4	11	17	9	4	1
B..............	0	1	4	7	12	6	0

The kernels from each ear were studied separately, and it was interesting to find that the kernels of each ear varied from lighter to darker red quite like the individual ears of a progeny. The kernels of each ear were kept and studied separately, and arranged into groups according to the color intensity of the individual kernels, with the results represented in table 3. The letters *a*, *b*, *c*, *d*, and *e* in table 3 represent color-intensity classes ranging from whitish to dark dilute-red.

TABLE 3. VARIATION IN COLOR INTENSITY OF ORANGE KERNELS FROM SAME EAR, RANGING FROM WHITISH, a, TO A DARK DILUTE-RED, j

Ear No.	Color-intensity classes										Number of kernels
	a	b	c	d	e	f	g	h	i	j	
1	—	—	—	14	79	164	103	19	—	—	
2	—	—	—	—	3	52	140	35	6	—	
3	—	—	—	—	—	9	38	89	47	—	
4	—	—	19	84	191	60	5	—	—	—	
5	—	—	—	16	87	175	76	17	—	—	
7	—	31	106	144	82	13	—	—	—	—	
8	11	144	180	85	24	—	—	—	—	—	
9	—	—	—	11	39	160	79	20	—	—	
10	—	—	—	29	109	245	64	10	—	—	
11	—	—	4	41	191	185	—	—	—	—	
12	—	—	—	25	84	159	50	22	—	—	
13	—	—	—	22	108	113	20	—	—	—	
15	—	—	28	130	95	14	—	—	—	—	
18	—	54	134	64	—	—	—	—	—	—	
19	—	—	—	11	58	100	74	35	—	—	
20	7	106	44	9	—	—	—	—	—	—	
21	—	—	—	—	12	102	164	81	43	—	
22	—	—	47	262	173	37	—	—	—	—	
23	—	—	12	59	124	71	14	—	—	—	

The quantitative variation in color intensity among the kernels of the same ear is definite proof that it is not caused either by modifying factors or by multiple factors, for the pericarp is maternal tissue and, so far as such factors are concerned, should be genetically the same throughout. Quantitative variations in the soma of an individual which are genetic must be due to corresponding changes in the genes.

The kernels of 25 ears, heterozygous for dilute-red and colorless pericarps, were studied separately for color changes to darker red and to lighter red, variegation, and colorless with the results tabulated in table 4. Each kernel was examined separately and the number and size of color changes constituting a

TABLE 4. COLOR CHANGES IN PERICARP OF MAIZE HETEROZYGOUS FOR DILUTE-RED PERICARP, DILUTE-RED COB, AND COLORLESS PERICARP RED COB

Ear No.	Darker dilute-red to red							Lighter dilute-red and variegated							Parallel and adjacent segments of darker and lighter pericarp			Number kernels with recorded color changes	Number kernels with no recorded color changes	Total number kernels
	Line	Band	1/8	1/4	1/2	3/4	1	Line	Band	1/8	1/4	1/2	3/4	1	Line	Band	1/8			
1	56	6	—	1	1	—	—	76	9	—	—	—	—	—	22	3	—	226	153	379
2	18	—	—	—	—	—	—	23	—	—	—	—	—	—	3	—	—	94	142	236
3	46	2	—	—	—	—	—	42	7	1	—	—	—	—	4	—	—	127	56	183
4	56	2	1	1	—	1	—	45	3	—	—	—	—	—	2	—	—	170	201	371
5	87	6	1	—	1	—	—	89	8	1	1	—	—	—	20	—	1	278	81	359
6	150	2	—	—	—	—	—	142	5	—	2	—	1	—	23	—	—	303	67	370
7	144	6	2	1	—	1	—	147	3	2	1	—	—	—	13	2	—	290	86	376
8	116	8	—	2	—	—	—	99	8	—	1	1	1	—	9	—	—	275	169	444
9	53	1	—	—	—	—	—	55	2	—	1	—	—	—	3	—	—	142	167	309
10	155	9	4	2	2	1	—	144	6	1	1	—	—	—	10	4	—	315	142	457
11	141	9	2	—	—	—	—	122	6	—	1	1	—	—	7	—	—	356	65	421
12	66	5	—	—	—	—	—	55	5	—	—	—	—	—	5	2	—	211	129	340
13	24	—	—	—	—	—	—	32	2	—	—	—	—	—	1	—	—	108	184	292
14	82	3	—	—	1	—	1	84	3	—	—	—	1	—	10	—	—	171	93	264
15	114	5	—	1	—	—	—	112	2	—	—	—	—	1	9	—	—	181	86	267
16	54	8	1	—	1	1	—	41	10	—	1	—	—	—	2	—	—	254	107	361
17	52	2	—	—	—	—	—	38	3	—	—	—	—	—	—	—	—	158	62	220
18	37	3	—	—	—	—	—	46	4	—	—	1	1	—	3	1	—	133	119	252
19	31	1	—	—	2	—	1	32	2	1	1	2	1	1	1	—	—	132	146	278
20	100	3	1	—	2	—	—	114	3	—	—	1	—	—	3	—	—	224	178	402
21	13	—	—	—	—	—	—	24	1	—	—	—	—	—	—	—	—	83	83	166
22	206	13	1	1	—	—	—	216	5	1	—	1	—	1	13	1	—	393	126	519
23	107	5	—	—	1	—	—	117	7	1	—	1	1	—	1	—	—	178	102	280
24	65	2	—	—	—	1	1	66	3	—	1	1	—	—	2	—	—	297	89	386
25	32	—	—	—	1	—	—	32	2	1	—	—	—	—	—	—	—	105	183	288
Total	2005	101	13	9	12	5	3	1993	109	9	11	9	6	2	166	13	1	5204	3016	8220

line, band, and larger areas of its surface, as indicated in the table, were recorded. On the 8220 kernels examined were found 2005 lines of darker and 1992 lines of lighter color, 101 bands of darker and 109 bands of lighter color, and approximately equal numbers of darker and lighter color changes extending over larger areas as given in the table. Altogether there were 2148 color changes to darker and 2136 color changes to lighter. These results are in close agreement with the 1:1 ratio expected.

Similarly, the kernels of each of 20 ears homozygous for dilute-red pericarp were studied individually for color changes to darker and lighter colors with the results given in table 6. The number and size of every color change covering a line and larger parts of a kernel were recorded for each kernel. On the 6191 kernels examined were found 551 lines of darker and 1505 lines of lighter color, 36 bands of darker and 112 bands of lighter color, and larger darker and lighter color changes in about the same proportion, as given in table 6. In all there were 600 color changes to darker and 1652 changes to lighter, a ratio of 1:2.75. This is in fairly close agreement with the 1:3 ratio expected.

Mention should be made of the fact that the homozygous dilute-red kernels were of a dark shade, and it is possible that color changes to lighter were more conspicuous than similar changes to darker red.

To determine whether the somatic segregation of darker and lighter colors in the ratio of 1:1 occurs generally in dilute-red pericarp heterozygous for colorless, a number of pedigreed ears of known genetic constitution were taken at random and the color changes recorded with the results given in table 5. In every ear the number of changes to darker and lighter colors was about the same, with a total of 471 changes to darker and 453 changes to lighter colors on the 898 kernels examined. Also the kernels of one ear homozygous for dilute-red pericarp had 133 changes to darker and 390 changes to lighter colors. Again the color changes in the heterozygous dilute-red pericarp occurred in the ratio of one darker to one lighter, and in the homozygous dilute-red pericarp in the ratio of one darker to three lighter.

TABLE 5. COLOR CHANGES IN MAIZE PERICARP (A) HETEROZYGOUS FOR DILUTE-RED PERICARP, AND (B) HOMOZYGOUS FOR DILUTE-RED PERICARP

Pedigree	Color changes expressed in fractional parts of the surface of a kernel: Darker red							Lighter red and variegated							Total kernels examined
	Line	Band	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	Line	Band	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	
A 5428–5	201	23	1	1	4	1	0	195	25	2	2	2	1	0	447
6634–5	66	6	1	1	1	0	0	65	7	1	0	1	0	0	225
5465–1	93	3	2	0	3	0	0	86	2	2	0	0	1	0	148
5382–2	59	2	0	2	1	0	0	57	1	0	1	1	1	0	78
Total	419	34	4	4	9	1	0	403	35	5	3	4	3	0	898
B 6634–18	120	9	0	0	2	1	1	367	19	0	0	4	0	0	434

In the variegation series which ranges from dark red through various shades of red and dilute-red to whitish, from heavy variegation to light variegation, to colorless, the lightest shades of dilute-red should change to variegations more

TABLE 6. COLOR CHANGES IN PERICARP OF MAIZE HOMOZYGOUS FOR DILUTE-RED PERICARP DILUTE-RED COB

Ear No.	Darker dilute-red to red						Lighter dilute-red and variegated						Parallel and adjacent segments of darker and lighter pericarp			Number kernels with recorded color changes	Number kernels with no recorded color changes	Total number kernels
	Line	Band	1/8	1/4	1/2	3/4	Line	Band	1/8	1/4	1/2	3/4	Line	Band	1/8			
1	33	—	—	—	—	—	90	4	—	1	—	—	1	—	—	151	118	269
2	17	1	1	—	—	—	44	6	—	—	—	—	3	2	—	94	204	298
3	8	3	—	—	—	—	16	1	—	—	—	—	—	—	—	51	188	239
4	13	—	—	—	1	—	47	—	—	—	—	—	2	—	—	54	143	197
5	11	—	1	—	—	—	49	1	—	—	—	—	1	—	—	68	232	300
6	64	7	—	2	—	—	120	19	2	2	1	1	5	3	—	214	84	298
7	42	2	—	1	—	—	76	8	1	—	—	—	6	1	1	149	186	335
8	81	3	1	1	—	—	270	14	2	2	1	—	32	3	—	381	153	534
9	18	4	—	—	—	—	44	11	1	—	—	—	5	—	—	214	127	341
10	14	—	—	—	—	—	45	3	—	—	—	—	—	—	—	59	159	218
11	34	—	—	—	—	—	92	4	1	—	—	—	4	1	—	154	228	382
12	17	2	—	—	—	—	34	6	1	1	—	1	3	—	—	94	303	397
13	36	1	—	—	—	—	123	2	1	1	—	—	9	—	—	176	209	385
14	36	2	—	—	—	—	107	3	2	2	—	—	2	1	—	143	238	381
15	19	2	—	1	—	—	53	2	—	—	—	—	—	—	—	74	186	260
16	6	—	—	—	—	—	10	3	—	—	—	—	—	—	—	19	140	159
17	4	6	1	—	—	—	17	4	—	—	—	—	—	—	—	45	173	218
18	44	3	2	1	—	—	93	13	4	2	—	—	4	—	—	209	147	356
19	44	—	—	—	—	—	137	5	1	3	—	—	6	—	—	159	120	279
20	10	—	—	—	—	—	38	3	1	—	—	—	2	—	—	79	266	345
Total	551	36	6	6	1	—	1505	112	17	14	2	2	85	11	1	2587	3604	6191

rapidly than the darker shades of dilute-red, and the reverse. Also the light dilute-reds should give rise to variegated areas extending over a whole kernel and larger patches, while such changes should be rare in the darker shades of dilute-red and in the reds. So far as materials are available, these expectations are realized. The heterozygous dilute-red ears of pedigree 6756 were divided into 3 groups according to their general color intensity. Ten ears were classed as medium dilute-red, 14 ears as light-medium dilute-red, and 6 ears as light dilute-red. The 10 medium dilute-red ears had 3 kernels that were entirely variegated, the 14 light-medium dilute-red ears had 11 variegated kernels, and the 6 light dilute-red ears had 73 variegated kernels.

If dominance among the genes for dilute-red and variegated pericarps tends to vary inversely as the number of the *a* elements in the gene structure, light dilute-reds should tend to be dominant over dark dilute-reds, light variegations should be dominant over heavy variegations, and variegations should be dominant over dilute-reds.

No data are available on the dominance relationships between dilute-reds of different color intensities.

There is some evidence that variegations are dominant over dilute-reds. Hayes ('17) intercrossed mosaics and dilute-reds (patterns) and found mosaic to be dominant over dilute-red. In my own cultures, pedigree 6756 segregated homozygous dilute-reds, heterozygous dilute-reds, and colorless in the ratio of 1:2:1. The homozygous dilute-reds had dilute-red cobs and the heterozygous dilute-reds had red cobs. One of the ears was variegated in cob and pericarp, in consequence of a change in the constitution of one of the dilute-red genes so that it produced a variegation instead of a dilute-red. It is less likely that both dilute-red genes changed coincidently to have a sufficiently low *a* element constitution to produce a variegation. The chances are 3:1 that the change occurred in one of the dilute-red genes only, and the fact that the pericarp is variegated instead of dilute-red indicates that the variegated condition is dominant over dilute-red. The more frequent occurrence of variegated kernels in homozygous than in heterozygous dilute-red pericarp is further evidence of the dominance of variegations over dilute-reds.

Such data as are now available on the dominance relationsips between variegations of different patterns were furnished by Emerson and are given in table 7. The variegated patterns are indicated by numbers ranging from 1, representing a very light pattern, to 7, representing a very heavy pattern. Colorless pericarp is indicated by a zero. Very light variegation crossed with colorless, indicated in the table by 1×0, gave an F_1 progeny of 40 ears with a mean grade of variegation of 5.25. Very light variegation crossed with the heaviest variegation, indicated in the table by 1×7, gave an F_1 progeny of 111 ears with a mean grade of variegation of 4.15. In these particular crosses the mean grade of variegation pattern is lighter when the extremely light and heavy patterns are intercrossed than when the extremely light pattern is crossed with colorless. The progenies of the other crosses given in table 7, with the exception of the cross 0×7, have mean grades of variegation pattern between 4 and 5,

regardless of the variegation grades of the parents. These data are inconclusive as to the dominance relationships of the different variegation patterns. It even can not be said that the F_1 patterns are intermediate between the parents. Further data and doubtless different methods will be necessary to determine these relationships.

Emerson ('17) pointed out as a striking feature of his records the fact that in a progeny from a homozygous medium-variegated ear the medium-variegated kernels did not give rise to a single very light-variegated ear, while the light-variegated kernels produced 18 very light-variegated ears. The occurrence of light-variegated kernels on a homozygous medium-variegated ear is evidence in favor of the dominance of the light over the medium pattern. The fact that the light-variegated kernels gave rise to very light-variegated ears while the medium-variegated kernels did not is in accordance with the general hypothesis.

TABLE 7. COMPARISON OF VARIEGATION PATTERNS OF PARENT AND PROGENY EARS (DATA FROM EMERSON)

Parent grades of variegation	Progenies—Number of ears								
	Grade of variegation*								Mean grade
	1	2	3	4	5	6	7	Total	
0 × 1	—	—	3	8	13	8	8	40	5.25
7 × 1	—	21	10	36	22	19	3	111	4.15
7 × 0	1	0	1	4	20	22	38	86	6.07
7(×)	—	—	—	7	3	3	8	21	5.57
0 × 2	5	18	63	29	55	33	8	211	4.14
7 × 2	—	2	20	42	4	16	1	85	4.17
2(×)	—	17	15	—	—	—	—	32	2.47
3(×)	—	7	6	6	1	—	2	22	3.41
4(×)	—	1	1	3	—	1	—	6	3.83
6(×)	—	—	—	2	—	—	2	4	5.50

* Grades of variegation are represented by numbers from 1, which represents very light variegation, to 7, which represents the heaviest pattern. Colorless is indicated by 9.

The relative frequencies of changes from one color or pattern to another as reported in my previous studies (Eyster '24, '25) are in entire accord with the expected results. In the calico type of variegation changes to self-color were found to increase from 2 per 1000 kernels in the light variegations to 87 per 1000 kernels in heavy variegations. In the mosaic type of variegation the heavy pattern was found to change to a medium pattern at the rate of 27 changes per 1000 kernels, and to light mosaic at the rate of 8 per 1000 kernels, and the medium pattern changed to light pattern at the rate of 19 changes per 1000 kernels. That is to say, the medium pattern changed to light pattern 1.9 times as often as the heavy pattern changed to light pattern.

It has been shown that somatic segregation of darker and lighter pericarp in the soma of an individual occurs in the relation of 1:1 in heterozygous and 1:3 in homozygous dilute-red pericarps. Intercrosses between dilute-red pericarps of different intensities, and between variegations of different patterns and dilute-reds of different intensities should give F_1 pericarps that would segregate

darker and lighter colors in different ratios depending upon the constitution of the genes which are associated in the respective crosses. In pericarp having associated genes which differ sufficiently so that each would produce a different effect if associated with a colorless gene, the dominant gene will individually determine the color changes to darker and lighter until by the chance assortment of its elements it comes to have the approximate constitution of its associated gene, when a change in the one, the other, or both will produce a color change in the direction of the dominant condition, but a coincident change and segregation in both genes will be necessary to produce a color change in the direction of the recessive condition. Data on the frequencies of color changes in the pericarp of such intercrosses, and on the relative frequencies of color changes in heterozygous and homozygous pericarp of all the different intensities of dilute-red and patterns of variegations should make it possible to determine the actual number of elements in a gene.

Gene Changes and Multiple Allelomorphs

A change in a gene which causes it to give expression to a character in contrast to the type form has been called a point mutation. Commonly point mutations involve a change from one extreme condition to another, as tall to dwarf stem, red to white pericarp or flower color, and starchy to sugary endosperm. Evidently, in such changes all of the elementary units of a gene which are in a certain physical, chemical, or physico-chemical condition, which may be represented by *a*, are changed by some influence to another condition, *b*. It may be supposed that such a change would give rise to an allelomorphic gene with the general stability of the original gene. It is quite possible that the elements might change from the *a* condition to a number of other conditions, as *c*, and *d*, according to the influence which causes it to change, thus giving rise to a series of multiple allelomorphs, which may or may not give rise to an orthogenetic series of quantitative changes.

It is not surprising, and is rather to be expected, that more or less frequently only some of the elements of a gene become changed from the *a* to the *b* condition, thus giving rise to a gene with 2 kinds of elements in its structure. Such a change would also be considered a point mutation. The contrasting gene elements of a gene of this sort reproduce themselves between each two mitoses and are assorted at random and segregated in the soma by the mechanism of mitosis, and thus give rise to such genetic quantitative changes as occur in variegations, and which have by others been called by such names as bud sports, mutations, somatic mutations, and sporophytic mutations. Since the variations of a variegation series are the result of the random assortment of gene elements in the soma, the segregation of associated genes in sporogenesis, and the recombination of genes in fertilization, it is obvious that they are distinctly different from point mutations. The genetic variations which result as a consequence of a point mutation in which some but not all of the elements are changed from one physical or chemical condition to another, so that gene elements for contrasting conditions are present in the same gene, might be called *secondary mutations*.

If a single gene element of the dominant condition, in a gene which is made up of elements for both the dominant and the recessive conditions, produces a visible effect in the soma, a quantitative series of variations will ultimately result ranging from the dominant to the recessive condition, but no variegations will be produced. Orthogenetic series of variations of this sort, caused by a series of multiple allelomorphs, are quite common in both plants and animals.

If a number of the gene elements for the dominant condition, in a gene made up of contrasting gene elements, are present in a gene without producing a visible effect in the soma, variegations will be produced in addition to the quantitative changes ranging from the dominant to the recessive condition. This is due to the fact that a particular gene with both kinds of gene elements may give expression to the recessive condition, but more or less frequently, depending upon the make-up of the gene, daughter genes will be produced with enough of the elements for the dominant condition to express it and thus will occur a somatic change from the recessive to the dominant condition.

Variegations may occur which do not include a quantitative series of types ranging from the dominant to the recessive condition in the allelomorphic series. A variegation gene, it will be recalled, has gene elements for both the dominant and the recessive conditions, but the one or the other condition will be expressed depending upon the relative numbers of the contrasting gene elements. If the minimum number of gene elements necessary to give expression to the dominant condition in the soma expresses it in its most intense condition, a series of variegations will result which does not include a series of quantitative variations ranging from the dominant to the recessive condition.

The conception that a gene may be made up of contrasting gene elements has an important bearing on the presence-and-absence theory. Multiple allelomorphs have been used by some geneticists as an argument against the theory, while others, especially Bateson ('26), argue that the occurrence of multiple allelomorphs amounts almost to a demonstration of the correctness of the presence-and-absence hypothesis. If the "presence" and "absence" are considered in a relative sense, the presence-and-absence hypothesis applies to the quantitative changes that occur in the genes which give rise to the variations in variegations and to all quantitative variations that are caused by the random assortment of gene elements within the gene. In maize pericarp the presence of a certain number of *a* elements and absence of a corresponding number of *b* elements gives rise to a dilute-red color of the pericarp and cob, while the presence of a smaller number of *a* elements and a correspondingly larger number of *b* elements produces a variegation in the manner already indicated.

Quantitative Inheritance

The popular conception among geneticists is that quantitative variations are due either to multiple factors or to a single factor with from one to many modifying factors, which may or may not be linked; but it must be admitted, in some instances, that the evidence for the one or the other of these interpretations is not well founded. This is especially true of the inheritance of size

differences, with the exception of such extreme variations as dwarfs. More recently it has been found that chromosome aberrations are often responsible for quantitative variations.

Gates ('17, 23) found size differences in the F_1 and later generations of crosses between *Oenothera biennis* and *Oenothera rubricalyx* which can not be satisfactorily explained on the basis either of multiple size factors or of modifying factors. The flowers of the F_1 plants from this cross were uniform and nearly intermediate in size as compared with the flowers of the parent species. The ranges of variability in the F_2 progenies were wider than the combined parent forms. The most striking feature of the F_2 plants was the variation in different flowers of the same plant, in different petals of the same flower, and even in different parts of the same petal. This type of variation does not fall within the definition of fluctuation but possesses an element of inheritance, as pointed out by Gates. Gates suggested that this type of variability stands between ordinary Mendelian inheritance on the one hand and fluctuation on the other, having certain features of both but differing from either in its irregularity.

The size variations described by Gates can be satisfactorily explained by assuming that they are due to a single gene made up of gene elements for different flower size, similar to the gene for variegated pericarp in maize. The differences in the size of flowers on the same plant, in size of petals in the same flower, and in size of different parts of the same petal are the expressions of genes having different numbers of the contrasting gene elements as a consequence of the random assortment of gene elements at the somatic mitoses. In brief, Gates was dealing with a variegation affecting size of petals which is fundamentally like the color variegation in maize pericarp. So far as can be determined from the descriptions, the size variations in the flowers of the *Oenothera* hybrids studied by Gates are of the same nature as the quantitative variations in color variegations, and are due to both Mendelian segregation and element segregation.

To what extent size inheritance in general is due to multiple factors, to a single factor with modifying factors, to chromosome duplications, or to a single gene which undergoes quantitative changes in its constitution in ontogenetic development as well as in sporogenesis, thus giving rise to a series of multiple allelomorphs, will have to be determined by suitable future experiments.

Summary

The common origin and allelomorphic relationships of a similar series of quantitative variations in all variegations that have been carefully studied indicate that a gene has undergone corresponding quantitative changes and that the fundamental nature of the gene changes is common to all variegations.

Somatic color changes occur in heterozygous dilute-red pericarp in the ratio of one change to darker red to one change to lighter red and colorless. In homozygous dilute-red pericarp, color changes appear to occur in the ratio of one change to darker red to three changes to lighter red and colorless. It follows that darker areas occur more frequently in heterozygous than in homozygous dilute-red pericarps.

A simple hypothesis has been advanced to account for the usually large series of multiple allelomorphs which produce an orthogenetic series of quantitative variations, and for the segregation of contrasting characters in the soma of individuals. The gene behaves as if it were made up of more elementary structural units, called *gene elements*.

When all of the gene elements of a gene change from one chemical, physical, or physico-chemical condition, as *a*, to some other condition, as *b*, an allelomorph is produced which has the general stability of the original gene. When only some of the elements change from an *a* to a *b* condition, a gene is created which has both kinds of elements in its constitution. By the random assortment of its elements a gene of this kind gives rise to a series of multiple allelomorphs which produce quantitative variations ranging from the dominant to the recessive condition, and to variegations if a number of the gene elements for the dominant condition may be present in a gene without producing a visible expression in the soma.

Some studies that have been made of quantitative inheritance could be more satisfactorily explained on the basis of gene-element segregation than as the result of multiple factors or modifying factors.

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MUTABLE GENES IN DROSOPHILA VIRILIS[1]

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Unstable genes, that is, those which mutate very frequently, have been known for a long time in plants, and now a large number of them have been described and their behavior analyzed.

Reddish body color. The first mutable gene in *Drosophila* was found in the spring of 1924, the reddish character being a sex-linked body color in *Drosophila virilis.* In a preliminary paper published recently (Demerec '26) a genetic analysis of the case was given, which makes it unnecessary to give the details in the present discussion.

The reddish gene is highly specific as far as the time of mutation is concerned. Reddish mutates back to wild type only in females, and only when the female is heterozygous, with one chromosome having reddish-1 and the other, one of its allelomorphs, that is, wild type, yellow or reddish-2. No reversions were found in homozygous females nor in males. Evidence indicates that the time of mutation is strictly limited to the maturation divisions. Mutations in somatic cells were not observed.

The mutable reddish gene is strikingly different from the majority of mutable genes found in plants. The majority mutate at all stages of the development, somatic mutations giving rise to somatic mosaics, which in the case of color are called variegations. There are, however, several cases known in plants in which mutations occur almost exclusively at the maturation divisions (Ikeno '23, '24, Imai '25). These cases are somewhat comparable to the reddish gene, which is still the most extreme case.

Miniature wings. In November of 1925, a second mutable gene was found in *Drosophila virilis.* This one is a sex-linked recessive, miniature wings. In this case, however, the gene mutates in males and females and also at all stages of the development. Somatic mutations produce the mosaics of different grades, and germinal mutations, together with early somatic mutations, give wild type individuals.

Easily distinguishable mosaics are very frequent in mutating miniature lines. When wings are examined under the low power microscope, flies can easily be found having one wing miniature and the other partly miniature and partly wild type, or having both wings mosaics of wild type and miniature tissues. The wings of *Drosophila virilis* are covered with hairs. There are approximately the same number of hairs present on the small miniature as on the large wild

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type wings. In the miniatures the hairs are located closer together. The closeness of the hairs can be used as a means of distinguishing small mutations, which could hardly be detected by the shape or size of the wing. Under high magnification small islands of wild type tissue can be observed in practically all mutating miniatures.

TABLE 1. THE FREQUENCY OF MUTATIONS OF REDDISH TO WILD TYPE, IMMEDIATELY AFTER THE ORIGIN OF REDDISH

Generation	Total number of flies	Number of reversions	Frequency of mutations, per cent[a]
2nd	241	30	24.8
3rd	9633	152	3.2
4th	8022	48	1.2
5th	2147	7	.62
6th	11532	5	.086
7th	4041	0	0.0

[a] Since the reddish gene was present in half of the flies only, the frequency of mutations was obtained by multiplying by two the percentage of reversions.

Mutable miniature wings can be compared in appearance and genetic behavior to better known cases of mutable variegations in plants.

Magenta eye color. In June of this year another mutable sex-linked character was found, that is, magenta eye color. The analysis, which is still in progress, shows that the mutations occur in both sexes, in somatic and also in germ cells. The case seems to be similar to that of mutable miniature wings.

The frequency of mutations at the time of the origin of the character. Both mutable reddish and miniature were found in experiments and were detected immediately after they originated. Fortunately, in the case of reddish, proper crosses were made at once and it was possible to measure the frequency of reversions beginning with the second generation of the existence of reddish. The data showing the frequency are given in table 1, from which it can be seen that the frequency of mutations rapidly decreased in each successive generation until reddish became practically constant in the fifth and sixth generations after its origin.

Mutable miniature was found as a single male. It was mated with several wild type females. All F_1 flies were wild type, but in F_2 instead of 25 per cent of total flies (50 per cent of males) being miniature, only 2.4 per cent of miniatures were obtained. A deficiency of miniatures was observed also in following F_2 generations and backcrosses, becoming, however, smaller and smaller in each successive generation (table 2). The deficiency was due to the reversions of gene for miniature to the gene for wild type. As can be seen from the data in table 2 the deficiency, that is, the frequency of mutations was very high immediately after the origin of the gene, becoming smaller and smaller in the following generations. The decrease in the frequency of mutations was especially rapid in backcrosses.

For several reasons mutable magenta character is not very satisfactory to work with, and the data on the frequency of mutations at the time of its origin were not obtained.

Occurrence of mutable genes. To detect the mutable property of genes in *Drosophila* it is necessary to make special tests, otherwise the chances are very high that they will be missed. Since the necessary technique was discovered five new mutations have been found in *Drosophila virilis*, three of them being mutable. Is there any significance in such a high proportion of mutable genes found among new mutations, or was it a matter of chance? Does it indicate the possibility that new mutations are potentially unstable at the time of their origin, becoming constant sooner or later afterwards? It is yet too early to draw any conclusion, but it is only a question of relatively short time to obtain more data on that point and come closer to solving it. It seems to me, however, that the evidence so far obtained has more than accidental significance.

TABLE 2. PERCENTAGE OF MINIATURES OBTAINED IN FIRST F_2 GENERATIONS AND BACKCROSSES

Generation	F_2 generations		Backcrosses	
	Total number of flies	Percentage of miniatures	Total number of flies	Percentage of miniatures
1st	540	2.4	—	—
2nd	10083	.84	956	.105
3rd	6093	4.5	233	15.
4th	4087	13.4	72	43.

A preliminary search through the literature gave an unexpectedly strong support to that view. Contrary to the case in Drosophilas, in plants the mutable condition of a gene could very easily be detected, especially if the gene mutates in somatic cells as well. An examination of Baur's ('24, '26, '26a) work on *Antirrhinum* revealed that about 50 per cent of new mutations described by him, which were adequately tested, were mutable. Ikeno ('23, '24) describes two mutations found in his cultures of *Plantago*, both of them mutable. As far as the data could be analyzed, all but probably one of the mutations described by Terao ('17, '22) and Nagai ('26, '26a, '26b, '26c) in rice were mutating. Especially interesting are the results obtained by Nagai. He finds, not only that almost all new mutations were mutable, but in two cases he also observed a large deficiency of mutant gametes in the first F_2 generations, that deficiency decreasing rapidly in the following generations. He observed apparently the same condition found in the case of reddish and miniature, that is, that the frequency of mutations was high at the time of the origin of the gene, decreasing rapidly in the following generations.

Since similar conditions to those found in *Drosophila virilis* were observed also in several plants, it is very likely that the high proportion of mutable genes found among the newly originated mutations is not an exception, but a fact which could be generally expected.

Discussion. A working hypothesis which assumes that the gene is not the smallest hereditary unit but a complex structure composed of smaller units could very well explain the conditions described in this paper. A complex-gene

hypothesis was formed by Correns (Correns '19) and E. G. Anderson[2] to explain the mutable condition of genes causing variegations in *Capsella* and maize respectively. The evidences presented here on the origin of mutations tend to generalize that hypothesis.

Summary. Three mutable genes are known in *Drosophila virilis*, that is, genes for:

Reddish body color which mutates only at the reduction divisions of heterozygous females, miniature wings and magenta eye color which mutate in all stages of development of females and males.

In two cases, which were fully tested, it was found that the frequency of mutations was high at the time of the origin of the new gene, decreasing rapidly in the following generations.

Out of five adequately tested new mutations three were found to be mutable.

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[2] Presented in a paper read at the meeting of the Genetic Section of Am. Soc. of Zoologists and Bot. Soc. of America at the Toronto meeting of Am. Assn. for Adv. of Sci., December 28, 1921.

AUTHOR AND SUBJECT INDEX, VOLUME I

Agriculture, and nature, in dry regions of U. S. S. R., 47
Algae, genetic relationships in, 457
Allelomorphs, multiple, versus multiple factors, 803
Allen, C. E. Influences determining appearance of sexual characters, 333
Angiosperm phylogeny, flower anatomy and, 423
Angiosperms, antiquity of, 429; phylogeny of, 413
Antiquity of the angiosperms, 429
Aquatic vegetation, dynamic factors affecting, 667
Arrhenius, O. Soil acidity and plant growth, 53
Associations, plant, and their classification, 629, 643
Bacteria, carbon and energy utilization in, 203; morphological and physiological variations in, 175; morphological variation and growth, 185; of Black and Asov Seas, 211
Bacterial taxonomy and nomenclature, 195
Bacteroid-like form and immunity in leguminous plants, 59
Bergstrand, H. Morphological and physiological variations in bacteria, 175
Biochemistry, oxidation-reduction, in relation to, 225
Blackburn, K. B. Occurrence of sex chromosomes in flowering plants, origin, 299
Blakeslee, A. F. The composition of Nubbin, a compound (2n+1) chromozome type in Datura, 831
Borodin, D. N. Introduction of cultivated plants into Russia, 171; Russian field crops in U. S., 61
Borza, A. Problems of insular floras and vegetations, 655
Botanico-geographical regions of Oaxaca, 525
Breeding, disease resistant varieties, 137; wheat for disease resistance, 149
Buchanan, R. E. Present status of bacterial taxonomy, 195
Buchholz, J. T. Embryogeny of conifers, 359
Cajander, A. K. Scientific foundation of forestry, forest research work in Finland, 769
Cappelletti, C. Bacteroid-like form and immunity in leguminous plants, 59
Carbon and energy utilization, by bacteria, 203
Chamberlain, C. J. Structural evidence for genetical relationships in vascular plants, 473
Chance as an element in plant geography, 591
Chodat, R. Morphological continuity as shown by a comparison of fossil and living plants, 487
Chromosome, numbers and taxonomy, 307; structure in mitosis and meiosis, 265
Chromosomes, number, in Ectocarpus virescens, 259
Clark, W. M. Oxidation-reduction, in relation to biochemistry, 225
Cleland, R. E. Meiosis in pollen mother cells of the Oenotheras, etc., 317
Climatic character of last interglacial period in Europe, 647
Climatic deterioration, postglacial, of northern Europe, 663
Communities, plant, of Juan Fernandez Islands, 565
Composition of nubbin, a compound (2n+1) chromosome type in Datura, 831
Concept of the gene, 889
Conifers, embryogeny of, 359; paleozoic, 401
Conzatti, C. Botanico-geographical regions of Oaxaca, 525
Cowles, H. C. The succession point of view in floristics, 687
Cultivated plants, origin of, 167
Cytology, of plant hybrids, 821; of Triticum in relation to genetics, 345
Demerec, M. Mutable genes in Drosophila virilis, 943

Dickson, J. G. (See Holbert, J. R., 155)
Dictyota, periodic fruiting of, 393
Disease resistance, breeding wheat for, 149
Disease resistant, strains of corn, 155; varieties, breeding, 137
Distribution of species, factors controlling, 673
Domin, K. Problems of plant ecology, 497
Drosophila virilis, mutable genes, in, 943
Dry regions of U. S. S. R., agriculture of, 47
Du Rietz, G. E. Factors controlling distribution of species in vegetation, 673; Fundamental units of vegetation, 623
Dynamic factors affecting aquatic vegetation, 667
Eames, A. J. Role of flower anatomy in determination of angiosperm phylogeny, 423
East, E. M. The concept of the gene, 889
Ecology, problems of, 497
Ectocarpus virescens, number of chromosomes in two different forms of, 259
Elaioplasts, nature and function of, 311
Embryogeny of conifers, 359
Energy utilization by bacteria, 203
Experimental, error in field plot trials, 107; results, statistics in interpretation of, 55
Eyster, W. H. Bearing of variegations on the nature of the gene, 923
Factors controlling distribution of species in vegetation, 673
Field crops, Russian, in U. S., 61
Field plot trials, experimental error in, 107
Florin, R. Paleozoic conifers, 401
Floristics, succession point of view in, 687
Flower anatomy, role of, in angiosperm phylogeny, 423
Flowering plants, phylogeny of, 413
Forest, experiment station development, problems in, 757; experiment stations, fundamental to scientific silviculture, 693; floor, light versus soil moisture in, 575; investigative work in Canada, 701; research in Finland, 769
Forests, of India, improvement and development of, 751; role of, in circulation of water on earth's surface, 741
Forestry, scientific foundations of, in Finland, 769
Fossil and living plants, morphological continuity of, 487
Fundamental units of vegetation, 623
Gene, as basis of life, 897; variegations, and nature of, 923; concept of, 889
Genes, mutable, in Drosophila virilis, 943
Geobotanical, research in Switzerland, present state, 603; studies in Spain, method and nomenclature, 451
Geographical (botanico-) regions of Oaxaca, 525
Gleason, H. A. Plant associations and their classifications, 643
Harper, R. A. Nature and functions of plastids, especially elaioplasts, 311
Hayes, H. K. Breeding disease resistant varieties, 137
Heilborn, O. Chromosome numbers and taxonomy, 307
Henrici, A. T. Morphological variation and growth in bacteria, 185
Holbert, J. R. and J. G. Dickson. Development of disease-resistant strains of corn, 155
Howe, C. D. Aspects of forest investigative work in Canada, 701
Hoyt, W. D. Periodic fruiting of Dictyota, 393
Hutchinson, J. Phylogeny of flowering plants, 413
Hybridizations in shepherd's purse, 837
Hybrids, plant, cytology of, 821; reciprocal, 787
Improvement of forests of India, 751
Increment, thinning and, 779
Initial root habit in American trees, 713

Institute of Applied Botany (in Russia), 161
Insular floras and vegetation, problems, 655
Interglacial period in Europe, climatic character of, 647
Introduction of cultivated plants into Russia, 171
Issatchenko, B. Bacteria of the Black and Asov Seas, 211; and A. Salimowska. Thiobacteria of salt water, 221
Jonson, T. Methods and aids in tree form investigations, volume, growth, etc., 729
Kiesselbach, T. A. Varietal, cultural, and seasonal effects upon water requirements, 87
Kirssanoff, A. T. Relation between plant growth and water table, 129
Lehmann, E. Reciprocal hybrids, 787
Leighty, C. E. Breeding wheat for disease resistance, 149
Levine, M. Influence of radium emanation on microsporogenesis of lily, 271
Linkage of a higher order, 833
Love, H. H. Statistics in the interpretation of experimental results, 55
Maize, disease resistance in, 155
Malinowski, E. A case of linkage of a higher order, 833.
Mechanism of mitotic division 243
Meiosis, in pollen mother cells of Oenotheras, 317; mitosis, chromosome structure in, 265
Method and nomenclature, in geobotanical studies in Spain, 541
Methods in tree form investigations, volume growth, etc., 729
Microsporogenesis in lily, influence of radium on, 271
Mitotic division, mechanism of, 243
Mitosis and meiosis, chromosome structure in, 265
Morphological, and physiological peculiarities of Podostemonaceae,351;and physiological variations in bacteria, 175; continuity, a comparison of fossil and living plants, 487; variation and growth in bacteria, 185
Movements, growth, 1
Muller, H. J. The gene as the basis of life, 897
Multiple allelomorphs versus multiple factors, 803
Munns, E. N. Problems in American forest experiment station development, 757
Mutable genes in Drosophila virilis, 943
Nature and agriculture in dry regions of U. S. S. R., 47
Nemec, B. Mechanism of mitotic division, 243
Nichols, G. E. Plant associations and their classification, 629
Nomenclature, bacterial, 195; geobotanical studies in Spain, 541
Nubbin, a compound chromosome type in Datura, 831
Oenotheras, meiosis in, 317
Origin of cultivated plants, 167
Oxidation-reduction, in relation to biochemistry, 225
Paleobotany and vascular anatomy, 481
Paleozoic conifers, 401
Palmgren, A. Chance as an element in plant geography, 591
Pathology, fifty years of, 13
Pavari, A. (See Serpieri, A., 693)
Pearsall, W. H. Dynamic factors affecting aquatic vegetation, 667
Periodic fruiting of Dictyota, 393
Petrini, S. Thinning and increment, 779
Phylogeny, flowering plants, 413; angiosperm, flowering anatomy, 423
Physiological and morphological peculiarities of Podostemonaceae, 351
Pistacia, self- and inter-fertility of, 815
Plant, associations and their classification, 629, 643; communities of Juan Fernandez Islands, 565; geography, chance as an element in, 591; growth, soil acidity and, 53; movements, 1
Plastids, nature and function of, 311
Podostemonaceae, morphological and physiological peculiarities of, 351

Pollen mother cells, meiosis in Oenotheras, 317
Postglacial period, and climatic deterioration of northern Europe, 663
Present state of geobotanical research in Switzerland, 603; of bacterial taxonomy and nomenclature, 195
Problems, in American forest experiment station development, 757; of insular floras and vegetation, 155; of plant ecology, 497
Protoplasmic structure, 251
Radium emanation, effect of, on microsporogenesis in lily, 371
Reciprocal hybrids, 787
Relation between plant growth and water table, 129
Rodger, A. Improvement and development of the forests of India, 751
Role of flower anatomy in determination of angiosperm phylogeny, 423; forests in circulation of water on earth's surface, 741
Root habit, initial, in American trees, 713
Rübel, E. Present state of geobotanical research in Switzerland, 603
Russian field crops in U. S., 61
Salimowska, A. (See Issatchenko, B., 221)
Salt water, thiobacteria of, 221
Savastano, G. Preliminary experiments in self- and inter-fertility of Pistacia, 815
Sax, K. Cytology of Triticum in relation to genetics, 345
Scientific foundations of forestry in Finland, 769
Seifriz, W. Protoplasmic structure, 251
Self- and inter-fertility of Pistacia, 815
Sernander, R. Warm postglacial period and the postglacial climatic deterioration of northern Europe, 663
Serpieri, A. and A. Pavari. Forest experiment stations, fundamental to scientific silviculture, 693
Sex chromosomes, occurrence in flowering plants, origin, 299
Sexual characters, influences determining, 333
Shepherd's purse, species hybridizations in, 837
Shull, G. H. Species hybridizations in shepherd's purse, 837
Silviculture, forest experiment stations and, 693
Sirks, M. J. Multiple allelomorphs versus multiple factors, 803
Skottsberg, C. Plant communities of the Juan Fernandez Islands, 565
Smith, E. F. Fifty years of pathology, 13
Soil acidity and plant growth, 53
Species hybridizations in shepherd's purse, 837
Stadler, L. J. Experimental error in field plot tests, 107
Statistics in interpretation of experimental results, 55
Structural evidences for genetic relationships, algae, 457
Succession, point of view in floristics, 687; the concept and its values, 677
Svedelius, N. Number of chromosomes in two different forms of Ectocarpus virescens, 259. Structural evidences for genetic relationships, algae, 457
Szafer, W. Climatic character of last interglacial period in Europe, 647
Tansley, A. G. Succession: The concept and its values, 677
Taylor, W. R. Chromosome structure in mitosis and meiosis, 265
Taxonomy, bacterial, 195; chromosome numbers and, 307
Thinning and increment, 779
Thiobacteria of salt water, 221
Thompson, R. B. Vascular anatomy and paleobotany, 481
Tischler, G. Cytology of plant hybrids, 821
Toumey, J. W. Initial root habit in American trees, 713. Vegetation of the forest floor; light versus soil moisture, 575
Tree form investigations, methods in, 729
Trees, initial root habit in, 713

Triticum, cytology of, in relation to genetics, 345
Tulaikov, N. M. Nature and agriculture in dry regions of U. S. S. R., 47
Variegations, bearing of on nature of the gene, 923
Varietal, cultural, and seasonal effects upon water requirements, 87
Vascular, anatomy and paleobotany, 481; plants, structural evidence for genetical relationships in, 473
Vavilov, N. I. Origin of cultivated plants, 167. The Institute of Applied Botany and of the Improvement of Cultivated Plants, 161
Vegetation, factors controlling species distribution in, 673; insular, 655
Villar, E. H. del. Method and nomenclature employed in geobotanical studies in Spain, 541
Waksman, S. A. Energy utilization and carbon assimilation by autotrophic bacteria, 203
Water requirements, varietal, cultural, and seasonal effects upon, 87
Water table, plant growth and, 129
Went, F. A. F. C. Morphological and physiological peculiarities of Podostemonaceae, 351. Plant movements, 1
Wieland, G. R. Antiquity of the angiosperms, 429
Zon, R. Rôle of forests in the circulation of water on earth's surface, 741